Aus dem Institut für Tropenmedizin und Internationale Gesundheit Berlin
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Die Rolle des Ernährungszustandes von Patienten mit Diabetes mellitus Typ 2 und Malaria in sub-Sahara Afrika

Zur Erlangung des akademischen Grades
Doctor rerum medicarum (Dr. rer. medic.)

vorgelegt der Medizinischen Fakultät Charité –
Universitätsmedizin Berlin

von Dipl. Ern.wiss. Ina Danquah, geb. Schöttle
aus Potsdam

Gutachter: 1. Prof. Dr. Frank P. Mockenhaupt
2. Prof. Dr. Olaf Müller
3. Prof. Dr. Klaus Stark

Datum der Promotion: 8. April 2011

meiner Oma

INHALTSVERZEICHNIS

ZUSAMMENFASSUNG

Die Rolle des Ernährungszustandes von Patienten mit Diabetes mellitus Typ 2 und Malaria in sub-Sahara Afrika

Abstract

Infektionskrankheiten sind noch immer die Hauptursache für Morbidität und Mortalität in Sub-Sahara Afrika. Gleichzeitig sieht sich diese Region einer wachsenden Prävalenz von nicht-übertragbaren Krankheiten gegenüber. Für die Anfälligkeit und das Ausmaß beider Erkrankungsformen kann der Ernährungszustand eine entscheidende Rolle spielen. Diese Arbeit hatte daher das Anliegen, die Wechselbeziehungen von Infektionskrankheiten, nicht-übertragbaren Erkrankungen und Ernährungszustand im Afrika südlich der Sahara zu charakterisieren.

Drei Studien aus Ghana widmen sich dieser Fragstellung: (i) Unterernährung und „Intermittierende Therapie von Malaria bei Kleinkindern“ (IPTi) [1-3], (ii) Eisenmangel und Malaria bei Schwangeren [4,5], und (iii) Diabetes mellitus Typ 2 (DM2) und Malaria-Infektion bei Erwachsenen [6].

In der ersten Studie aus Nordghana erwies sich IPTi mit Sulfadoxin-Pyrimethamin bei 1200 Kleinkindern als effektive und verträgliche Möglichkeit der Malariakontrolle [1,3]. Bei den Kindern mit Malaria-Erkrankung zeigten sich in den ersten zwei Lebensjahren leichte Wachstumsverzögerungen. IPTi konnte jedoch nicht zu einer Verbesserung des kindlichen Wachstums beitragen. Der Ernährungszustand beeinflusste die Effektivität von IPTi: Bei unterernährten Kindern war diese halbiert ($P = 0,049$) [2].

In einer zweiten Studie wurde der Einfluss von Eisenmangel auf die Malaria bei Schwangeren untersucht. Sowohl Eisenmangel als auch Malaria führen zu Anämie. Daher werden Malariaprophylaxe und Eisensupplementierung in der Schwangerschaft empfohlen. Es gab allerdings Befürchtungen, Eisenpräparate könnten das Risiko einer Malaria-Infektion erhöhen. Diese Hypothese wurde indirekt durch die Beobachtungen bei 530 Schwangeren aus Zentralghana gestützt: Eisenmangel reduzierte die Prävalenz der Infektion um 33% ($P = 0,04$) [4].

Der dritte Aspekt zur Bedeutung von DM2 für das Malaria-Infektionsrisiko wurde in einer Fall-Kontroll-Studie zu Risikofaktoren für DM2 und Hypertonie in Zentralghana bearbeitet. Klinische und sozio-ökonomische Parameter sowie Ernährungsverhalten und Aktivitätsniveau von 1466 Studienteilnehmern wurden erhoben und analysiert. Wie erwartet war DM2 mit dem Ernährungszustand assoziiert. Darüber hinaus konnte in dieser Studie erstmals gezeigt werden, dass mehr DM2-Patienten als Kontrollpersonen mit *P. falciparum* infiziert waren (16% vs. 10%; $P = 0,001$). Im multivariaten Modell entsprach das einer Risikosteigerung für eine Malaria-Infektion um 46% ($P = 0,02$) [6].

Diese Ergebnisse unterstreichen die Notwendigkeit weiterer Untersuchungen zum Zusammenhang von Ernährungszustand und Malaria, um fokussierte Ernährungsinterventionen und spezifische Präventionsprogramme für Malaria-Risikogruppen in SSA zu entwickeln.

Einleitung

Malaria fordert jährlich bis zu 3 Millionen Menschenleben weltweit [7]. Vor allem Kinder und Schwangere in Afrika sind davon betroffen. Dort stützt sich die Malaria-Kontrolle auf die frühe Diagnose und eine rechtzeitige Behandlung. Jedoch hat der Großteil der Bevölkerung nur begrenzten Zugang zu den notwendigen formalen Strukturen des Gesundheitssystems [8].

Bei Kleinkindern versucht die „Intermittierende Therapie von Malaria“ (IPTi) diese Versorgungslücke zu schließen. Sie nutzt die funktionierende Infrastruktur des Immunisierungsprogramms EPI (Expanded Program on Immunization): Zu den regulären Impfterminen wird den Kindern unabhängig von einer bestehenden Infektion mit dem Erreger der Malaria tropica, *Plasmodium falciparum,* eine kurative Einzeldosis Sulfadoxin-Pyrimethamin (SP) verabreicht [1]. Für die zweite Malaria-Risikogruppe der Schwangeren empfiehlt die Weltgesundheitsorganisation (WHO) gleichermaßen eine intermittierende Malariatherapie (IPTp) im Rahmen der Vorsorgeuntersuchungen [9,10]. Sowohl IPTi als auch IPTp scheinen viel versprechende Strategien der Malariakontrolle für Sub-Sahara Afrika (SSA) zu sein. IPTi-SP reduziert im ersten Lebensjahr die Episoden klinischer Malaria um 30% und das Risiko einer Anämie um 21% [1]. IPTp mindert die Risiken für schwere Anämie in der Schwangerschaft (39%), niedriges Geburtsgewicht (40%) und neonatale Sterblichkeit (61%) [11,12].

Neben den Infektionskrankheiten sind Protein-Energie-Mangelernährung (PEM) und Mikronährstoff-Defizienzen wie Eisenmangel häufige Krankheitsursachen in SSA. PEM ist dort mit 50% [13,14] aller Todesfälle bei unter 5-Jährigen assoziiert. Eisenmangel während der Schwangerschaft erhöht das Risiko für Komplikationen, niedriges Geburtsgewicht und Frühgeburtlichkeit [15]. Sowohl PEM als auch Eisenmangel stehen in Zusammenhang mit Malaria. Es gibt Anhaltspunkte für eine verminderte Effektivität von Malariamedikamenten bei unterernährten Kindern [16-18] sowie für ein erhöhtes Malariarisiko bei der Eisensupplementierung von Schwangeren [19]. Für eine zuverlässige Beurteilung sind weitere epidemiologische Daten erforderlich.

Zusätzlich belastet ein sprunghafter Anstieg von nicht-übertragbaren Erkrankungen die Länder Afrikas. Im Jahr 2030 werden dort rund 24 Millionen Erwachsene allein an der Stoffwechselkrankheit Diabetes mellitus (DM) leiden [20,21]. Bekanntermaßen sind Diabetiker anfällig für Infektionen der Atmungsorgane, der Harnwege und der Haut [22]. Ein möglicher Zusammenhang von DM mit tropischen Krankheiten wie Malaria kann in Afrika erhebliche Auswirkungen haben. Dies wurde jedoch bislang nur wenig untersucht.

Zielstellung

Diese Arbeit charakterisiert die Wechselbeziehungen von Malaria, Ernährungszustand und Diabetes mellitus Typ 2 (DM2) in Ghana anhand von drei Studien. Folgende Ziele wurden bearbeitet:

- Der Einfluss von Malaria auf den Ernährungszustand Kleinkindern
- Die Wirkung von IPTi-SP auf das Wachstum von Kleinkindern
- Die Effektivität von IPTi-SP in Abhängigkeit vom Ernährungszustand bei Kleinkindern
- Die Bedeutung von Eisenmangel für das Infektionsrisiko mit *P. falciparum* bei Schwangeren
- Die Bedeutung von DM2 für das Infektionsrisiko mit *P. falciparum* bei Erwachsenen

Methoden

Studie I: IPTi und Unterernährung bei Kleinkindern

Studienort und -design
Die IPTi-Studie wurde im semi-urbanen Nordghana durchgeführt. Die Region ist hyperendemisch für Malaria mit mäßig saisonalen Schwankungen. Bettnetze wurden zur Zeit der Studie kaum genutzt (3%). Malaria wurde hauptsächlich mit Chloroquin behandelt (Heilungsraten <50%) [17]. Nahezu jedes vierte Kind leidet an Unterernährung [23].
Die Studie wurde randomisiert, doppelt verblindet und Plazebo-kontrolliert durchgeführt [3]. Sie untersuchte die Effektivität von IPTi bei 1200 Kleinkindern. Jeweils 600 Kinder erhielten eine halbe Tablette SP oder Plazebo im Alter von 3, 9 und 15 Monaten. Zu allen Untersuchungsterminen (im Alter von 3, 6, 9, 12, 15, 18, 21 und 24 Monaten) wurden klinische Daten erhoben und eine Anamnese aufgenommen. Eine venöse Blutprobe wurde entnommen; Fieber (T ≥37,5°C) wurde axillar gemessen. Eine Infektion mit Malariaparasiten wurde mikroskopisch diagnostiziert und die Hämoglobin-Konzentration (Hb) wurde photometrisch bestimmt (HemoCue, Ångelholm, Schweden). Malaria lag vor bei Parasitämie *plus* akutem Fieber oder Fieber während der letzten 48h. Schwere Anämie wurde definiert als Hb <7 g/dl und schwere Malaria nach WHO-Kriterien [24]. Malaria wurde mit Artesunat (Plasmotrim; Mepha, Schweiz) behandelt. Andere Erkrankungen wurden nach den Richtlinien des Ghana Health Service kuriert [25]. Weitere Informationen zum Studienaufbau und -ablauf sind in den entsprechenden Publikationen [1,3] nachzulesen.

Messgrößen für Unterernährung
Unterernährung wurde anhand anthropometrischer (Größe, Gewicht) und demographischer Parameter (Alter, Geschlecht) nach aktuellen WHO-Referenzen definiert (WHOAnthro Software, Version 2.0.2) [26]. Zur Klassifizierung der Kinder in Wohl- und Unterernährte wurden statische Kategorisierungen und ein dynamisches (nicht-parametrisches) Mischmodell verwendet [27]. Die Details sind in Publikation [2] beschrieben.

Datenverarbeitung und statistische Analyse
Zunächst wurde der Einfluss des Ernährungszustandes auf die Haupt-Endpunkte (asymptomatische Parasitämie, unkomplizierte Malaria, schwere Malaria, schwere Anämie, und Tod) anhand von Inzidenzdichten (ID) mittels negativer Binomialregression ermittelt (Inzidenz-Raten-Ratios, IRR). Umgekehrt wurde der Einfluss von Erkrankungen auf die Entwicklung der anthropometrischen Z-Scores unter Verwendung von *Generalized Estimating Equation* (GEE) untersucht. Weiterhin wurde der Effekt von IPTi auf die körperliche Entwicklung (Gewichtszunahme, Längenwachstum, Z-Score-Zunahme) mittels Mann-Whitney-U Test analysiert. Die Hauptanalyse befasste sich mit dem Vergleich protektiver Effektivitäten (PE = 1-IRR) von IPTi zwischen Unterernährten und Wohlernährten mittels negativer Binomialregression zu unterschiedlichen Beobachtungs-zeiträumen. Signifikante Unterschiede wurden mittels Wald-Test ermittelt. Die statistische Auswertung ist in Publikation [2] detailliert erklärt.

Studie II: Malaria und Eisenmangel bei Schwangeren

Studienort und -design
Während einer Querschnittstudie zu Malaria in der Schwangerschaft wurden 530 Schwangere im semi-urbanen Zentral-Ghana rekrutiert. Die Region ist hyper- bis holoendemisch für Malaria [5]. Die Teilnehmerinnen wurden klinisch untersucht, die Schwangerschaftswoche bestimmt sowie periphere Blutproben (EDTA) und Urinproben gesammelt. Fieber wurde

definiert als axillare Temperatur ≥37,5°C. Malariaparasiten wurden im Dicken Tropfen mikroskopisch ausgezählt. Zusätzlich wurden geschachtelte *P. falciparum*-spezifische PCR-Verfahren nach DNS-Extraktion aus stabilisiertem Blut angewendet (AS1-Puffer; QIAmp, Qiagen, Deutschland) [28]. Chloroquin und Pyrimethamin im Urin wurden mittels ELISA nachgewiesen.

Messgrößen für Eisenmangel-Anämie
Die Hämoglobin-Konzentration (Hb) wurde photometrisch bestimmt (HemoCue, Ångelholm, Schweden) und Anämie definiert als Hb <11 g/dl. Serum-C-reaktives Protein (CRP) wurde mittels Immunoturbidimetrie (Biokit, Deutschland) und Serum-Ferritin mittels ELISA (IBL, Deutschland) quantifiziert. Vier Definitionen wurden für Eisenmangel herangezogen: (i) Ferritin <12 ng/ml [29], (ii) Ferritin <12 ng/ml oder Ferritin <51 ng/ml, wenn CRP >6 ng/ml [30], (iii) Ferritin <30 ng/ml [31], und (iv) Ferritin <30 ng/ml oder Ferritin <70 ng/ml, wenn CRP >8,2 ng/ml [32].

Datenverarbeitung und statistische Analyse
Geometrische Mittelwerte der Parasitendichten (GMPD) wurden durch $\log_{10}$-Transformation ermittelt und 95% Konfidenzintervalle (95% KI) berechnet. Gruppenvergleiche metrischer Variablen wurden mittels t-Test, ANOVA, Mann-Whitney-U Test und Kruskal-Wallis Test je nach Anwendbarkeit durchgeführt. χ^2-Test wurde für Vergleiche kategorialer Parameter genutzt. Risikoschätzer für *P. falciparum*-Infektion (Odds Ratio, OR) wurden durch logistische Regression ermittelt und für Störvariablen adjustiert.

Studie III: Diabetes und Malaria bei Erwachsenen

Studienort und -design
Im urbanen Zentral-Ghana wurde eine Fall-Kontroll-Studie zur Identifizierung von Risikofaktoren für DM2 und Hypertonie von Juni 2007 bis Juli 2008 durchgeführt. Die Teilnehmer wurden im Diabetes-Zentrum, der Hypertonie-Klinik, bei Bekannten, Freunden und Nachbarn dieser Patienten sowie aus dem Krankenhauspersonal rekrutiert. Weitere Einzelheiten zur Rekrutierung sind in Publikation [6] dargelegt.

Die Patienten wurden zu Krankheitsvorgeschichte, sozio-ökonomischem Hintergrund, Aktivitätsniveau und Ernährungsverhalten befragt. Sie wurden körperlich untersucht und um die Abgabe von Blut- und Urinproben gebeten. Hb wurde photometrisch gemessen (B-Hemoglobin; HemoCue, Ångelholm, Schweden). Im Routinelabor wurden *P. falciparum* Infektionen durch mikroskopische Auszählung festgestellt. Die DNS wurde aus stabilisiertem Blut extrahiert (AS und QIAmp, Qiagen, Deutschland) und spezifische PCR-Verfahren zur Detektion der Malaria-Parasiten angewendet [28].

Messgrößen für DM2
Die Nüchternglukose-Konzentration (NG) wurde photometrisch ermittelt (Glucose 201^+, HemoCue, Ångelholm, Schweden). DM2 wurde definiert als dokumentierte Anti-Diabetes-Therapie und/oder NG ≥7 mmol/l [33]; Hypertonie als bekannte Anti-Hypertonie-Therapie und/oder Blutdruck ≥140/90 mmHg [34]. Kontrollen waren negativ für beide Befunde.

Datenverarbeitung und statistische Analyse
Verteilungsunterschiede metrischer und kategorialer Variablen wurden jeweils mittels Mann-Whitney-U-Test und χ^2-Test oder Fishers Exakt Test identifiziert. Risikoschätzer (OR) und 95% Konfidenzintervalle (95% KI) wurden durch logistische Regression ermittelt und gegebenenfalls für Störvariablen adjustiert.

Ergebnisse

Studie I: IPTi und Unterernährung bei Kleinkindern

Studienpopulation
Zwischen März und September 2003 erhielten 1200 Kleinkinder randomisiert SP oder Plazebo. Die Gruppen unterschieden sich nicht in ihren Basisdaten, Abbrecherquoten und Mortalitätsraten [3]. Neunundachtzig Prozent der Kinder erhielten alle drei Dosen IPTi, und 87% wurden erfolgreich bis zum 24. Monat nachbeobachtet. Zu den Monaten 3, 9 und 15 waren jeweils 32%, 40% and 50% unterernährt. Die Basisdaten der unterernährten und nicht unterernährten Kinder unterschieden sich nicht.

Interaktion zwischen Ernährungszustand und Malaria
Für den gesamten Beobachtungszeitraum hatte der Ernährungszustand keinen Einfluss auf die IDs asymptomatischer Parasitämie und unkomplizierter Malaria. Jedoch erhöhte Unterernährung das Risiko zu versterben um 89% (95% KI, 3-247%) und chronische Unterernährung das Risiko für schwere Anämie um 49% (95% KI, 7-107%). Umgekehrt verschlechterten Parasitämie und Malaria die Messgröße für chronische Unterernährung (height-for-age, HAZ). Dieser Einfluss auf HAZ war besonders ausgeprägt hinsichtlich schwerer Anämie und wurde auch für Untergewicht (weight-for-age, WAZ) beobachtet.

Einfluss von IPTi auf das kindliche Wachstum
Gewichtszunahme, Längenwachstum, Zunahme des Oberarmumfangs und der Z-Scores wurden analysiert. IPTi verbesserte die Gewichtszunahme lediglich im ersten Lebensjahr und nur in geringem Ausmaß. Außerdem war dieser Effekt nur bei Kindern zu beobachten, die die ersten zwei Dosen IPTi in einem guten Ernährungszustand erhielten. Sowohl im 2. Lebensjahr als auch im Gesamtzeitraum (3-24 Monate) konnte kein positiver Effekt von IPTi auf das Wachstum festgestellt werden.

Tabelle 1. Protektive Effektivitäten (%) von IPTi bei unterernährten und wohl ernährten Kindern

Endpunkt	**Protektive Effektivität (%) von IPTi**					
	1. Lebensjahr		2. Lebensjahr		3-24 Monate	
	Wohl-ernährte	Unter-ernährte	Wohl-ernährte	Unter-ernährte	Wohl-ernährte	Unter-ernährte
Parasitämie	52,8	16,0	26,6	14,6	24,7	10,9
Klinische Malaria	46,0	**25,2***	18,6	-4,5	18,4	9,5
Schwere Anämie	48,2	-31,8	21,3	**10,2***	22,7	16,8

*, Unterernährte vs. Wohlernährte; P <0.05 nach Wald-Test

Effektivität von IPTi bei Unterernährung
Die PEs von IPTi wurden berechnet für den jeweiligen Ernährungszustand und adjustiert für Regenzeit und Nahrungsmittelverfügbarkeit. Eine signifikante Reduktion des Risikos durch IPTi wurde nur bei gut ernährten Kindern beobachtet. Insgesamt waren die PEs bei den Unterernährten ungefähr halbiert im Vergleich zu den Wohlernährten (Tabelle 1). Obwohl

diese Unterschiede zumeist nicht signifikant waren, war die PE für Malaria bei unterernährten Kindern im ersten Lebensjahr signifikant niedriger als bei wohlernährten (25% vs. 46%; *P* = 0,049). Ähnliches wurde auch für schwere Anämie nach der zweiten Dosis bzw. im 2. Lebensjahr festgestellt. Sowohl die Betrachtung in 6-Monats-Abständen als auch das nicht parametrische Mischmodell bestätigten verringerte PEs bei Unterernährten. Besonders auffällig waren die PEs für schwere Malaria. Obwohl Unterernährte und Wohlernährte gleiche IDs aufwiesen (je 0,03/Jahr), zeigte sich eine Tendenz zur Protektion nur bei Wohlernährten. Unterernährte Kinder der SP-Gruppe schienen sogar anfälliger für schwere Malaria zu sein als die der Plazebo-Gruppe (14 Episoden vs. 5 Episoden; *P* = 0,04 Wald Test).

Studie II: Malaria und Eisenmangel bei Schwangeren

Studienpopulation

Von 530 Schwangeren (Alter, 26,6 ± 6,3 Jahre) wiesen 44% (284/530) eine Anämie auf (Hb <11 g/dl). Bei 32% der Frauen konnten Malaria-Parasiten mikroskopisch nachgewiesen werden. Weitere 31% wiesen submikroskopische Infektionen auf, so dass sich eine Gesamtinfektionsrate von 63% ergab. Alle Proben zeigten *P. falciparum*-Infektion, bis auf eine mit *P. ovale*. Malaria-Medikamente, Chloroquin und Pyrimethamin, wurden bei 65% im Urin nachgewiesen [5].

Tabelle 2. Eisenmangel und Malaria bei 530 Schwangeren in Zentralghana

	Ferritin-Konzentration			
	<12 ng/ml [a]	<12 ng/ml oder <51 ng/ml, wenn CRP >6 ng/ml [b]	<30 ng/ml [c]	<30 ng/ml oder <70 ng/ml, wenn CRP >8.2 ng/ml [d]
Eisenmangel	5% (26/527)	18% (97/527)	26% (136/527)	38% (200/527)
Malariarisiko aOR (95% KI)	0,22 (0,09-0,54)	1,01 (0,63-1,60)	0,33 (0,22-0,50)	0,67 (0,46-0,98)

aOR, Odds Ratio adjustiert für Gravidität, Medikamentenspiegel
[a], nach Cook [29]; [b], nach Kuvibidila [30]; [c], nach van den Broek [31];
[d], nach Kabyemela [32]
Für drei Schwangere gab es keine Ferritin-Konzentrationen.

Eisenmangel und Malaria

Die Serum-Ferritin-Konzentrationen lagen zwischen 2 und 968 ng/ml. Anämische Patientinnen zeigten höhere Spiegel als nicht-anämische (70,8 vs. 45,7 ng/ml; *P* <0,001), und *Plasmodium*-infizierte Schwangere hatten höhere Werte als nicht infizierte (70,8 vs. 40,6; *P* <0,001). Die Prävalenzen von Eisenmangel anhand vier unterschiedlicher Definitionen sind in Tabelle 2 zusammengestellt. Nach der Definition von Kabyemela *et al.* [32] präsentierten sich 38% mit Eisenmangel. Von diesen waren 113 Frauen PCR-positiv für *P. falciparum*. Nach Einbeziehung von Gravidität und Medikamentenspiegeln ergab sich eine Risikoreduktion für Malaria bei Eisenmangel um 33% (95% KI, 2-54%). Im Vergleich dazu zeigten sich mikroskopisch nachweisbare Infektionen bei 21,5% der Frauen mit Eisenmangel, wohingegen die Infektionsrate bei Patientinnen ohne Eisenmangel 38,8% (OR, 0,43; 95% KI, 0,28-0,66) betrug. Bei Verwendung der Definitionen nach Cook [29] und van den Broek [31] zeigten sich ebenfalls protektive Effekte für *P. falciparum*-Infektion, nicht jedoch bei der Klassifizierung nach Kuvibidila [30] (Tabelle 2).

Studie III: Diabetes und Malaria bei Erwachsenen

Studienpopulation
Von den 1466 Teilnehmern wiesen 46% einen DM2 auf. Die mittlere NG betrug 8,3 mmol/l (Spanne, 1,3-37,1 mmol/l). Siebenundneunzig Prozent (655/675) nahmen Antidiabetika (524, Metformin; 412, Sulfonylharnstoffe; 160, Glitazone; 148, Insulin). Dennoch hatte nahezu die Hälfte (317) erhöhte NG. Die Patienten ohne DM2 wurden als Gesamtgruppe betrachtet, obwohl sich gesunde Kontrollen (n = 377) und Kontrollen mit Hypertonie (n = 414) hinsichtlich ihrer demographischen und sozio-ökonomischen Parameter teilweise unterschieden. Die NG war jedoch ähnlich zwischen diesen beiden Gruppen (4,5 ± 0,7 vs. 4,6 ± 0,8 mmol/l; P = 0,53). DM2 Patienten waren älter (54,7 ± 13,4 vs. 47,1 ± 15,9 Jahre; P <0.001), hatten einen geringeren sozio-ökonomischen Status (z.B., fehlende Schulbildung, 35,7% vs. 16,5%; P <0,001) und rauchten häufiger (7,3% vs. 4,4%; P = 0,024) als Teilnehmer ohne DM2.

Malaria
Nur 0,9% (13/1466) aller Studienteilnehmer hatten eine mikroskopisch nachweisbare Infektion mit Malaria-Parasiten. Die entsprechende mittlere Parasitendichte war mit 880/µl niedrig (Spanne, 80-4960/µl). Mit spezifischen PCR-Verfahren wurde eine Infektionsrate von 14,1% festgestellt. *P. falciparum* wurde in 91,7% dieser Infektionen nachgewiesen. Keiner der infizierten Teilnehmer hatte Fieber. Der mittlere Hb war jedoch um 0,4 g/dl reduziert verglichen mit nicht infizierten Teilnehmern (P = 0,004).

Diabetes und Malaria
Bei Patienten mit DM2 wurden mehr *Plasmodium*-Infektionen beobachtet als bei nicht diabetischen Teilnehmern (Prävalenz von *P. falciparum*, 16% vs. 10%; P = 0,001). Diese Beobachtung konnte nicht durch unterschiedliche Antimalaria-Medikationen zwischen den Gruppen erklärt werden (Diabetiker, 7 vs. Nicht-Diabetiker, 13; P = 0,32). Es gab auch keinen Hinweis auf ein erhöhtes *P. falciparum*-Risiko durch Antidiabetika: 14,1% der DM2 Patienten mit Metformin und 26,0% ohne Metformin waren infiziert (P = 0,01). Kontrollen und Hypertonie-Patienten zeigten vergleichbare Infektionsraten mit *P. falciparum* (9,3% vs. 11,2%; P = 0,38).

In einem multiplen Regressionsmodell für *P. falciparum*-Infektion erhöhte DM2 adjustiert für Alter und Geschlecht das Risiko für diese Infektion um 46% (95% KI, 6-103%; P = 0,02). Weitere unabhängig mit einer *P. falciparum*-Infektion assoziierte Faktoren waren ein Wohlstandswert <25. Perzentile (aOR, 1,76; 95% KI, 1,27-2,42) und Analphabetismus (aOR, 1,59; 95% KI, 1,11-2,28). Für die univariat assoziierten Parameter Wohnort, Ethnizität, Schulbildung, Beruf und Haushaltsgröße wurde zuvor Multikollinearität identifiziert, so dass diese aus dem Modell ausgeschlossen wurden.

Wenn DM2 im beschriebenen Modell durch NG ersetzt wurde, ergab sich eine Risikosteigerung für *P. falciparum* mit jedem mmol/l Blutglukose um 5% (95% KI, 2-9%; P = 0,002). Das war sowohl nominal bei Nicht-Diabetikern (aOR, 1,23; P = 0,19) als auch signifikant bei Diabetikern nachzuweisen (aOR, 1,04; P = 0,02). Bei DM2 Patienten wurde NG ≥8,6 mmol/l als kritischer Schwellenwert für ein erhöhtes *P. falciparum*-Risiko identifiziert (aOR, 1,63; 95% KI, 1,06-2,53; P = 0,03).

Diskussion

Unterernährung und Malaria sind weiterhin die wichtigsten Ursachen für Morbidität und Mortalität in SSA [7,14]. Zusätzlich sieht sich dieser Kontinent einem rasanten Anstieg chronischer und ernährungsbedingter Erkrankungen gegenüber [20]. Der Zusammenhang von Ernährungszustand und Malaria wurde in drei epidemiologischen Studien in Ghana untersucht. Die erste Studie zeigt eine verminderte Effektivität des Malaria-Präventionsprogramms IPTi-SP bei unterernährten Kleinkindern [2]. Im Gegensatz dazu reduziert Eisenmangel das Risiko für *P. falciparum*-Infektion bei Schwangeren [4]. Letztlich weisen überernährte, diabetische Erwachsene ein erhöhtes Infektionsrisiko mit Malaria-Parasiten auf [6].

Bislang wurde IPTi-SP als einfaches, gut verträgliches und kostengünstiges Malaria-Kontrollprogramm angesehen [1,3,35-39]. Allerdings ist Unterernährung bei afrikanischen Kleinkindern extrem häufig und kann bei Malaria-Therapie den Behandlungserfolg, die Aufnahmefähigkeit des Medikaments und die Immunantwort beeinträchtigen [16-18,40-43]. Es ist daher nicht überraschend, dass der protektive Effekt von IPTi-SP bei Unterernährung reduziert ist. Der Mechanismus für diesen Befund ist jedoch unklar. Vermeintlich höhere Inzidenzen für Malaria bei unterernährten Kindern wurden in unserer Studie nicht beobachtet und können als Ursache ausgeschlossen werden. Es gibt allerdings Hinweise darauf, dass Unterernährung die spezifische Immunität mindert [41,44]. IPTi-SP scheint ein gewisses Maß an Immunabwehr gegen *P. falciparum* zu induzieren [45,46]. Ist der Ernährungszustand schlecht, wird die Protektion also abgeschwächt. Auch die Parasiten-Eliminierung könnte eingeschränkt oder verlangsamt sein [47]. Das Behandlungsversagen einiger Malaria-Medikamente bei unterernährten Kindern stützt diese Hypothese [18,40,48,49]. Die Pharmakokinetik von SP bei Unterernährten ist ebenfalls zu diskutieren. Erhöhte Ausscheidung, verminderte Medikamentenspiegel und reduzierte Halbwertzeit, wie für Chinin und Chloroquin beobachtet [42,43], würden die Bioverfügbarkeit und die Effektivität senken.

Die spezifische Konstellation von schwerer Malaria, IPTi und Unterernährung war grenzwertig signifikant. Die meisten Fälle schwerer Malaria wurden im Alter zwischen 16 und 24 Monaten beobachtet und hauptsächlich durch schwere Malaria-Anämie (Hb <5 g/dl) bestimmt [3]. Folsäuremangel, eine häufige Begleiterscheinung von PEM [50], könnte dafür verantwortlich sein. Er führt zu megaloblastischer Anämie [51]. SP stört den Folsäure-Metabolismus des Malaria-Parasiten [52], der wiederum in der Lage ist, externes Folat aufzunehmen [53]. Letztlich könnte die Gabe von SP bei bereits latentem Folsäuremangel zur Ausbildung einer schweren Anämie beitragen [54]. Die Konzentrationen von Tetrahydrofolat im Blut sind äußerst instabil und konnten in dieser Studie nicht bestimmt werden. Weitere Untersuchungen sind daher notwendig, um diese Hypothese zu prüfen.

Einerseits zeigen diese Ergebnisse ein unerkannt hohes Potential von IPTi bei wohl ernährten Kindern [3]. Andererseits ist der Wert eines Malaria-Kontrollprogramms für eine Region fragwürdig, in der seine Effektivität bei bis zu 80% der Zielgruppe geschwächt ist. Eine internationale Nahrungsmittelkrise könnte diesen Zustand weiterhin verschlechtern. Selbst bei bestehenden Ernährungsprogrammen ist unklar, ob und wann die Kinder von IPTi profitierten. Das wirft wiederum die Frage nach Einfachheit und Erschwinglichkeit dieser Strategie auf, wenn sie nicht in EPI integriert werden kann. Diese operationalen Probleme sollten Hauptanliegen bei der bevorstehenden Implementierung von IPTi-SP bei afrikanischen Kleinkindern sein. Gleichzeitig betonen die Ergebnisse dieser Studie erneut [23] die Notwendigkeit von Ernährungsprogrammen für die erfolgreiche Bekämpfung von Malaria in SSA. Flächendeckende Schulungen für die Früherkennung von Unterernährung und für

Ernährungsberatung, die Aufklärung der Eltern und Ernährungsprogramme sind dringend erforderlich.
Die Ergebnisse der zweiten Studie zu Eisenmangel und Malaria lassen weniger deutliche Schlussfolgerungen zu. Die Charakterisierung von Eisenmangel in Malaria-Endemiegebieten ist problematisch. Bei entzündlichen Prozessen reagiert Serum-Ferritin als Akutphase-Protein und wird verstärkt in der Leber gebildet und freigesetzt. Ohne die Einbeziehung von CRP kann Ferritin nicht eindeutig interpretiert werden. Ein verbindlicher Grenzwert für CRP zur Beurteilung des Ferritin-Spiegels ist jedoch nicht festgelegt [29]. Die Anwendung unterschiedlicher Werte beeinflusst die Stärke der Assoziation von Eisenmangel und Malaria (Tabelle 2). Dieser Umstand verdeutlicht die Schwierigkeit, Eisenmangel bei Schwangeren mit *P. falciparum* oder anderen Infektionskrankheiten zuverlässig zu diagnostizieren. Die entsprechenden Verfahren sollten genau evaluiert werden. Weiterhin sind dringend Studien notwendig, um die Anwendung von Eisenpräparaten bei Schwangeren in Malaria-Endemiegebieten zu beurteilen.

Mit der dritten Studie wurde zum ersten Mal ein erhöhtes Risiko für die Infektion mit Malaria-Erregern bei diabetischen Patienten gezeigt. Die genauen Ursachen für diesen Befund sind zwar unklar, jedoch ist ein erhöhtes Infektionsrisiko bei diabetischen Patienten durchaus biologisch plausibel. Beispielsweise könnten die Abwehrmechanismen gegen Blut- und/oder Leberstadien der Parasiten bei DM2-Patienten beeinträchtigt sein [22]. Die Persistenz der Parasiten wäre somit verlängert. Auch könnte eine verminderte T-Zell-Antwort bei nahezu unverändert humoraler Immunantwort involviert sein [55]. *In-vitro*-Studien beschreiben weiterhin, dass das Wachstum von *P. falciparum* durch hohe Glukoseverfügbarkeit angeregt wird [56]. Es ist zudem denkbar, dass Diabetiker durch die Produktion von z.B. Ketonkörpern attraktiver für den Vektor *Anopheles* als Nicht-Diabetiker werden. Die Mücken sind geleitet vom Geruchssinn und werden besonders von Ketongerüchen angezogen [57,58].

Obwohl die in dieser Studie erfassten *P. falciparum*-Infektionen asymptomatisch verliefen und nur submikroskopisch bestimmbar waren, kann die beobachtete Risikosteigerung bei bestimmten Patienten-Gruppen klinisch relevant werden. Dazu gehören Patienten mit fortgeschrittenem DM2. Es ist möglich, dass ihre Semi-Immunität geschwächt und damit die Kontrolle der Parasitenlast eingeschränkt ist [22,55]. Dafür spricht die festgestellte Risikosteigerung mit zunehmender NG. Im Gegensatz dazu könnten Kinder mit fehlender Semi-Immunität und Typ 1 Diabetes mellitus besonders anfällig für Malaria sein. Ähnliches ist auch anzunehmen für Frauen mit Gestationsdiabetes, da ihre Immunantwort auf *P. falciparum* der eines Immun-naiven gleicht [59]. Zudem sind diese leichten Malaria-Infektionen bei Diabetikern in Endemiegebieten ein bislang unerkanntes Infektionsreservoir [60]. Das Ausmaß von Malaria bei Diabetes mellitus in SSA sowie Relevanz und Ursachen dieses Zusammenhangs bedürfen weiterer Untersuchungen. Durch das rasante Fortschreiten von DM2 in dieser Region sind viele Afrikaner möglicherweise einem erhöhten Risiko für die *Plasmodium*-Infektion und Malariaerkrankung ausgesetzt.

Schlussfolgerung

Die hier dargestellten Studien zeigen exemplarisch, welchen Herausforderungen die Gesundheitspolitik in SSA gegenübersteht. Zum einen wird die Komplexität von Malariakontrollprogrammen bei Kleinkindern herausgestellt, die womöglich ohne gleichzeitige Ernährungsinterventionen und nachhaltige Verbesserungen nicht den gewünschten Erfolg erzielen. Zum anderen belegt die zweite Studie, dass eine Ernährungsintervention bei Schwangeren in Malaria-Endemiegebieten nicht in jedem Fall den Gesundheitszustand verbessert. Allein die biochemische Diagnostik von Mangelerkrankungen wie Eisenmangel ist dort problematisch. Eindeutige Ergebnisse zu Effektivität und Nebenwirkungen diesbezüglicher Interventionen lassen sich demzufolge nur schwer ermitteln. Zusätzlich sind nun die Auswirkungen nicht-übertragbarer Erkrankungen wie DM2 in Malaria-Endemiegebieten zu berücksichtigen. Erfolgreiche Ernährungsprogramme für die DM2-Prävention und -Therapie in SSA benötigen verstärkt die gesundheits- und finanzpolitische Aufmerksamkeit.

In den ressourcenarmen Ländern Afrikas gewinnt die interdisziplinäre Zusammenarbeit im Gesundheitswesen daher an großer Bedeutung. Nur so können zielgruppenorientierte, krankheitsspezifische und dabei einfache, gut verträgliche und kostengünstige Strategien zur Verbesserung der Gesundheit in der Bevölkerung entwickelt werden.

Literatur

1. Aponte JJ, Schellenberg D, Egan A, et al. (2009) Efficacy and safety of intermittent preventive treatment with sulfadoxine-pyrimethamine for malaria in African infants: a pooled analysis of six randomised, placebo-controlled trials. Lancet 374:1533-42
2. Danquah I, Dietz E, Zanger P, et al. (2009) Reduced efficacy of intermittent preventive treatment of malaria in malnourished children. Antimicrob Agents Chemother 53:1753-9
3. Mockenhaupt FP, Reither K, Zanger P, et al. (2007) Intermittent preventive treatment in infants as a means of malaria control: a randomized, double-blind, placebo-controlled trial in northern Ghana. Antimicrob Agents Chemother 51:3273-81
4. Danquah I, Bedu-Addo G, Mockenhaupt FP. (2008) Iron deficiency and Plasmodium falciparum infection during pregnancy. J Infect Dis 198:1573-4
5. Mockenhaupt FP, Rong B, Gunther M, et al. (2000) Anaemia in pregnant Ghanaian women: importance of malaria, iron deficiency, and haemoglobinopathies. Trans R Soc Trop Med Hyg 94:477-83
6. Danquah I, Bedu-Addo, G., Mockenhaupt, F.P. (2010) Increased risk of malarial infections in diabetes mellitus type 2. Emerging Infectious Diseases accepted 09 July 2010:
7. Breman JG. (2001) The ears of the hippopotamus: manifestations, determinants, and estimates of the malaria burden. Am J Trop Med Hyg 64:1-11
8. Breman JG, Alilio MS, Mills A. (2004) Conquering the intolerable burden of malaria: what's new, what's needed: a summary. Am J Trop Med Hyg 71:1-15
9. WHO/UNICEF/UNU. Iron deficiency anaemia: assessment, prevention and control: a guide for programme managers. Document WHO/NHD/01.3. Geneva, Switzerland: WHO, 2001.
10. Menendez C, D'Alessandro U, ter Kuile FO. (2007) Reducing the burden of malaria in pregnancy by preventive strategies. Lancet Infect Dis 7:126-35
11. Kayentao K, Kodio M, Newman RD, et al. (2005) Comparison of intermittent preventive treatment with chemoprophylaxis for the prevention of malaria during pregnancy in Mali. J Infect Dis 191:109-16
12. Shulman CE, Dorman EK, Cutts F, et al. (1999) Intermittent sulphadoxine-pyrimethamine to prevent severe anaemia secondary to malaria in pregnancy: a randomised placebo-controlled trial. Lancet 353:632-6
13. Katona P, Katona-Apte J. (2008) The interaction between nutrition and infection. Clin Infect Dis 46:1582-8
14. Pelletier DL, Frongillo EA, Jr., Schroeder DG, et al. (1995) The effects of malnutrition on child mortality in developing countries. Bull World Health Organ 73:443-8
15. Allen LH. (2000) Anemia and iron deficiency: effects on pregnancy outcome. Am J Clin Nutr 71:1280S-4S
16. Hess FI, Nukuro E, Judson L, et al. (1997) Anti-malarial drug resistance, malnutrition and socio-economic status. Trop Med Int Health 2:721-8
17. Mockenhaupt FP, Ehrhardt S, Eggelte TA, et al. (2005) Chloroquine-treatment failure in northern Ghana: roles of pfcrt T76 and pfmdr1 Y86. Ann Trop Med Parasitol 99:723-32
18. Wolday D, Kibreab T, Bukenya D, et al. (1995) Sensitivity of Plasmodium falciparum in vivo to chloroquine and pyrimethamine-sulfadoxine in Rwandan patients in a refugee camp in Zaire. Trans R Soc Trop Med Hyg 89:654-6
19. Oppenheimer SJ. (2001) Iron and its relation to immunity and infectious disease. J Nutr 131:616S-633S; discussion 633S-635S
20. International Diabetes Federation. IDF Diabetes Atllas 2009. 4th ed. Available from http://www.diabetesatlas.org, accessed 20 June 2010.
21. Mbanya JC, Kengne AP, Assah F. (2006) Diabetes care in Africa. Lancet 368:1628-9
22. Muller LM, Gorter KJ, Hak E, et al. (2005) Increased risk of common infections in patients with type 1 and type 2 diabetes mellitus. Clin Infect Dis 41:281-8
23. Ehrhardt S, Burchard GD, Mantel C, et al. (2006) Malaria, anemia, and malnutrition in african children--defining intervention priorities. J Infect Dis 194:108-14
24. World Health Organization. (2000) Severe falciparum malaria. Trans R Soc Trop Med Hyg 94:1-90
25. Ghana Health Service. Standard Treatment Guidelines, 5th ed., Ghana National Drugs Programme. Accra, Ghana, 2004.
26. World Health Organization. WHO Child Growth Standards - Methods and Development. World Health Organization. Geneva, Switzerland, 2006.
27. Aitkin M. (1999) A general maximum likelihood analysis of variance components in generalized linear models. Biometrics 55:117-28
28. Snounou G, Viriyakosol S, Zhu XP, et al. (1993) High sensitivity of detection of human malaria parasites by the use of nested polymerase chain reaction. Mol Biochem Parasitol 61:315-20
29. Cook JD, Skikne BS. (1989) Iron deficiency: definition and diagnosis. J Intern Med 226:349-55
30. Kuvibidila S, Yu LC, Ode DL, et al. (1994) Assessment of iron status of Zairean women of childbearing age by serum transferrin receptor. Am J Clin Nutr 60:603-9
31. van den Broek NR, Letsky EA, White SA, et al. (1998) Iron status in pregnant women: which measurements are valid? Br J Haematol 103:817-24
32. Kabyemela ER, Fried M, Kurtis JD, et al. (2008) Decreased susceptibility to Plasmodium falciparum infection in pregnant women with iron deficiency. J Infect Dis 198:163-6
33. World Health Organization. Definition, diagnosis and classification of diabetes mellitus and its complications: report of a WHO consultation. Part 1: diagnosis and classification of diabetes mellitus. Geneva, 1999.
34. World Health Organization/International Society of Hypertension. (1999) Guidelines for the management of hypertension. J Hypertens 17:151-183
35. Chandramohan D, Owusu-Agyei S, Carneiro I, et al. (2005) Cluster randomised trial of intermittent preventive treatment for malaria in infants in area of high, seasonal transmission in Ghana. Bmj 331:727-33

36. Grobusch MP, Lell B, Schwarz NG, et al. (2007) Intermittent preventive treatment against malaria in infants in Gabon--a randomized, double-blind, placebo-controlled trial. J Infect Dis 196:1595-602
37. Kobbe R, Kreuzberg C, Adjei S, et al. (2007) A randomized controlled trial of extended intermittent preventive antimalarial treatment in infants. Clin Infect Dis 45:16-25
38. Macete E, Aide P, Aponte JJ, et al. (2006) Intermittent preventive treatment for malaria control administered at the time of routine vaccinations in Mozambican infants: a randomized, placebo-controlled trial. J Infect Dis 194:276-85
39. Schellenberg D, Menendez C, Kahigwa E, et al. (2001) Intermittent treatment for malaria and anaemia control at time of routine vaccinations in Tanzanian infants: a randomised, placebo-controlled trial. Lancet 357:1471-7
40. Hamel MJ, Holtz T, Mkandala C, et al. (2005) Efficacy of trimethoprim-sulfamethoxazole compared with sulfadoxine-pyrimethamine plus erythromycin for the treatment of uncomplicated malaria in children with integrated management of childhood illness dual classifications of malaria and pneumonia. Am J Trop Med Hyg 73:609-15
41. Schaible UE, Kaufmann SH. (2007) Malnutrition and infection: complex mechanisms and global impacts. PLoS Med 4:e115
42. Treluyer JM, Roux A, Mugnier C, et al. (1996) Metabolism of quinine in children with global malnutrition. Pediatr Res 40:558-63
43. Walker O, Dawodu AH, Salako LA, et al. (1987) Single dose disposition of chloroquine in kwashiorkor and normal children--evidence for decreased absorption in kwashiorkor. Br J Clin Pharmacol 23:467-72
44. Shankar AH. (2000) Nutritional modulation of malaria morbidity and mortality. J Infect Dis 182 Suppl 1:S37-53
45. Greenwood B. (2007) Intermittent preventive antimalarial treatment in infants. Clin Infect Dis 45:26-8
46. Schellenberg D, Menendez C, Aponte JJ, et al. (2005) Intermittent preventive antimalarial treatment for Tanzanian infants: follow-up to age 2 years of a randomised, placebo-controlled trial. Lancet 365:1481-3
47. Francis D, Nsobya SL, Talisuna A, et al. (2006) Geographic differences in antimalarial drug efficacy in Uganda are explained by differences in endemicity and not by known molecular markers of drug resistance. J Infect Dis 193:978-86
48. MacArthur J, Stennies GM, Macheso A, et al. (2001) Efficacy of mefloquine and sulfadoxine-pyrimethamine for the treatment of uncomplicated Plasmodium falciparum infection in Machinga District, Malawi, 1998. Am J Trop Med Hyg 65:679-84
49. Muller O, Garenne M, Kouyate B, et al. (2003) The association between protein-energy malnutrition, malaria morbidity and all-cause mortality in West African children. Trop Med Int Health 8:507-11
50. Neumann CG, Swendseid ME, Jacob M, et al. (1979) Biochemical evidence of thiamin deficiency in young Ghanian children. Am J Clin Nutr 32:99-104
51. Whitney E, Rolfes, SN. The water-soluble vitamins: B vitamins and vitamin C. Understanding nutrition, 6th ed. St. Paul, MN: West Publishing, 1993;311-315.
52. Peters W. Chemotherapy and drug resistance in malaria. London, United Kingdom: Academic Press, 1997.
53. Dzinjalamala FK, Macheso A, Kublin JG, et al. (2005) Blood folate concentrations and in vivo sulfadoxine-pyrimethamine failure in Malawian children with uncomplicated Plasmodium falciparum malaria. Am J Trop Med Hyg 72:267-72
54. Uche-Nwachi EO. (1998) Effect of intramuscular sulfadoxine-pyrimethamine on pregnant Wistar rats. Anat Rec 250:426-9
55. Joshi N, Caputo GM, Weitekamp MR, et al. (1999) Infections in patients with diabetes mellitus. N Engl J Med 341:1906-12
56. Jensen MD, Conley M, Helstowski LD. (1983) Culture of Plasmodium falciparum: the role of pH, glucose, and lactate. J Parasitol 69:1060-7
57. Dalton P, Gelperin A, Preti G. (2004) Volatile metabolic monitoring of glycemic status in diabetes using electronic olfaction. Diabetes Technol Ther 6:534-44
58. Takken W, Knols BG. (1999) Odor-mediated behavior of Afrotropical malaria mosquitoes. Annu Rev Entomol 44:131-57
59. Fried M, Nosten F, Brockman A, et al. (1998) Maternal antibodies block malaria. Nature 395:851-2
60. Okell LC, Ghani AC, Lyons E, et al. (2009) Submicroscopic infection in Plasmodium falciparum-endemic populations: a systematic review and meta-analysis. J Infect Dis 200:1509-17

ANTEILSERKLÄRUNG

Frau Ina Danquah hatte folgenden Anteil an den vorgelegten Publikationen:

a) peer-reviewed Publikationen

Publikation 1:

Danquah I, Bedu-Addo G, Mockenhaupt FP. Increased risk of malarial infection in diabetes mellitus. *Emerg Infect Dis.* **2010**, accepted 09.07.2010

80 Prozent

Beitrag im Einzelnen:

- Planung des Forschungsvorhabens
- Patientenrekrutierung und Datenerhebung
- Datenaufbereitung und statistische Auswertung
- Verfassen der Publikation

Publikation 2:

Aponte JJ, Schellenberg D, Egan A, Breckenridge A, Carneiro I, Critchley J, **Danquah I**, Dodoo A, Kobbe R, Lell B, May J, Premji Z, Sanz S, Sevene E, Soulaymani-Becheikh R, Winstanley P, Adjei S, Anemana S, Chandramohan D, Issifou S, Mockenhaupt FP, Owusu-Agyei S, Greenwood B, Grobusch MP, Kremsner PG, Macete E, Mshinda H, Newman RD, Slutsker L, Tanner M, Alonso P, Menendez C. Efficacy and safety of intermittent preventive treatment with sulfadoxine-pyrimethamine for malaria in African infants: a pooled analysis of six randomised, placebo-controlled trials. *Lancet*. **2009**; 374. Epub 2009 Sep 17

10 Prozent

Beitrag im Einzelnen:

- Datenaufbereitung und statistische Auswertung der beteiligten Studie aus Tamale, Ghana

Publikation 3:

Danquah I, Dietz E, Zanger P, Reither K, Ziniel P, Bienzle U, Mockenhaupt FP. Reduced efficacy of intermittent preventive treatment of malaria in malnourished children. *Antimicrob Agents Chemother*. **2009** May;53(5):1753-9. Epub 2009 Feb 17

80 Prozent

Beitrag im Einzelnen:

- Analysen- und Methodenplanung
- Datenaufbereitung und statistische Auswertung
- Verfassen der Publikation

b) <u>nicht peer-reviewed Publikationen</u>

Publikation 4:

Danquah I, Bedu-Addo G, Mockenhaupt FP. Iron deficiency and *Plasmodium falciparum* infection during pregnancy. *J Infect Dis*. **2008**;198(10):1573-4.

50 Prozent

Beitrag im Einzelnen:

- Datenaufbereitung und statistische Auswertung
- Verfassen der Publikation

…… ……..

Ort, Datum Antragstellerin: Dipl. Ern.-wiss. Ina Danquah

…… ……..

Ort, Datum Betreuer: Prof. Dr. Frank P. Mockenhaupt

Type 2 Diabetes Mellitus and Increased Risk for Malaria Infection

Ina Danquah, George Bedu-Addo, and Frank P. Mockenhaupt

A case–control study of 1,466 urban adults in Ghana found that patients with type 2 diabetes mellitus had a 46% increased risk for infection with *Plasmodium falciparum*. Increase in diabetes mellitus prevalence may put more persons at risk for malaria infection.

In sub-Saharan Africa, infectious diseases remain the predominant cause of illness and death. *Plasmodium falciparum* malaria alone causes an estimated 1 million deaths annually (*1*). At the same time, sub-Saharan Africa faces the world's highest increase in type 2 diabetes mellitus; adaptation to Western lifestyles and genetic predispositions may accelerate this trend (*2*,*3*). A decade ago, type 2 diabetes mellitus prevalence in urban Ghana was 6.3% (*4*). By 2030, ≈20 million affected persons may live in sub-Saharan Africa (*2*). Type 2 diabetes mellitus increases susceptibility to common infections (*5*). In sub-Saharan Africa, the emerging co-occurrence of type 2 diabetes mellitus and tropical infectious diseases thus may have substantial implications. We describe prevalence of malaria infection in adults with and without type 2 diabetes mellitus residing in Kumasi, Ghana. Malaria transmission in Kumasi is low but patchy; mosquito breeding sites also occur in urban agricultural areas (*6*).

The Study

A case–control study of risk factors for type 2 diabetes and hypertension was conducted from August 2007 through June 2008 at Komfo-Anokye Teaching Hospital, Kumasi, Ghana. The patients' clinical and biochemical signs and symptoms were secondary objectives (I. Danquah et al., unpub. data). The study protocol was approved by the Ethics Committee, University of Science and Technology, Kumasi, and participants gave informed written consent.

Author affiliations: Institute of Tropical Medicine and International Health, Berlin, Germany (I. Danquah, F.P. Mockenhaupt); and Kwame Nkrumah University of Science and Technology, Kumasi, Ghana (G. Bedu-Addo)

DOI: 10.3201/eid1610.100399

Patients attending the diabetes (n = 495) or hypertension center (n = 451) were recruited. These patients promoted participation as preliminary (i.e., to be confirmed) controls to community members, neighbors, and friends (n = 222). Further preliminary controls were recruited from the outpatient department (n = 150) and among hospital staff (n = 148).

Participants were told to fast, abstain from alcohol and nicotine use, and avoid stressful and physical activities beginning at 10:00 PM the day before examination. On the day of examination, participants were asked about medical history and socioeconomic background, underwent physical examination, and provided venous blood and urine samples for laboratory testing.

Fasting plasma glucose (hereafter referred to as glucose concentration; fluoride plasma at 4°C) and hemoglobin (Hb) concentrations were measured (Glucose-201$^+$, B-Hemoglobin; HemoCue, Angelhom, Sweden). Irrespective of symptoms, malaria parasites were counted per 500 leukocytes on Giemsa-stained thick blood films. *Plasmodium* infection and species were ascertained by PCR that included positive and negative controls (*7*).

Patients with type 2 diabetes mellitus were defined as those receiving documented treatment with antidiabetes medication or having a glucose concentration ≥7 mmol/L (*8*); patients with hypertension were defined as those receiving documented antihypertension treatment or having mean blood pressure ≥140/90 mm Hg for 3 measurements (*9*). Controls had neither condition.

Between-group comparisons were performed by the Mann-Whitney U, χ^2, and Fisher exact tests. Logistic regression produced adjusted odds ratios (aORs), and 95% confidence intervals (CIs).

Of the 1,466 study participants, 675 (46%) had type 2 diabetes (Table 1). Among these, 655 (97.0%) received antidiabetes treatment, but 317 (47.0%) had increased glucose concentration (≥7 mmol/L). The 414 patients with hypertension but not diabetes and 377 controls with neither condition were grouped despite differences, e.g., in age and socioeconomic parameters (data not shown); however, glucose concentration was similar for the 2 groups (mean 4.51 vs. 4.56 mmol/L; p = 0.53).

According to microscopic examination, 13 (0.9%) of all participants had malaria parasites at low density (median 880/μL, range 80–4,960/μL). Reexamination by PCR showed that 206 (14.1%) were infected with *Plasmodium* spp., largely *P. falciparum* (189, 12.9%). Infected persons were afebrile, but mean hemoglobin was reduced (–0.4 g/dL; p = 0.004).

More *Plasmodium* spp. infections were observed in persons with type 2 diabetes mellitus than in those without the disease (Table 1); most infections were caused by *P. falciparum* (16% vs. 10%; p = 0.001). This difference was

Table 1. Demographic and clinical characteristics of 1,466 urban residents of Kumasi, Ghana, 2007–2008*

Characteristics	Persons with type 2 diabetes mellitus, n = 675	Persons without diabetes, n = 791	p value
Age, y, mean (range)	54.7 (18–92)	47.1 (18–100)	<0.0001
Male gender	171 (25.3)	182 (23.0)	0.299
Wealth score <25th percentile†	265 (39.6)	271 (34.3)	0.044
Illiteracy	308 (45.8)	206 (26.1)	<0.0001
Formal education, none	240 (35.7)	130 (16.5)	<0.0001
Crowded living condition‡	177 (26.7)	120 (15.3)	<0.0001
Smoking, current or quit	49 (7.3)	35 (4.4)	0.024
Akan ethnicity	592 (87.8)	685 (86.6)	0.480
Residence			
Kumasi metropolitan area	476 (70.8)	603 (76.2)	
Kumasi suburbs	174 (25.9)	162 (20.5)	
Elsewhere§	22 (3.3)	26 (3.3)	0.048
Occupation			
Public servant	44 (6.5)	194 (24.6)	
Trader	198 (29.5)	190 (24.1)	
Farmer	65 (9.7)	48 (6.1)	
Unemployed	248 (36.9)	138 (17.5)	
Other¶	117 (17.4)	218 (27.7)	<0.0001
FPG, mmol/L, mean (range)	8.3 (1.3–37.1)	4.5 (2.9–7.0)	<0.0001
Hemoglobin, g/dL, mean (range)	12.9 (5.8–19.1)	13.6 (4.9–19.1)	<0.0001
Fever, ≥37.5°C	2 (0.3)	4 (0.5)	0.693
History of fever, preceding week	95 (14.1)	93 (11.8)	0.182
Respiratory tract infection	5 (0.7)	11 (1.4)	0.232
Urinary tract infection#	14 (2.1)	7 (0.9)	0.076
Plasmodium spp. infection, by microscopy	5 (0.7)	8 (1.0)	0.582
Parasite density, per µL, median (range)	1,160 (160–2,480)	860 (80–4,960)	0.770
Plasmodium spp. infection, by PCR			
Plasmodium spp.	117 (17.4)	89 (11.3)	0.001
P. falciparum	108 (16.0)	81 (10.3)	0.001
P. malariae	14 (2.1)	9 (1.1)	0.205
P. ovale	8 (1.2)	7 (0.9)	0.611

*Values are no. (%) unless otherwise indicated. p values were calculated by Mann-Whitney U test or Fisher exact test, as applicable. FPG, fasting plasma glucose concentration.
†<25th percentile of a calculated index of 11 markers of wealth: electricity, pipe-borne water, radio, fan, cupboard, television, bicycle, motorbike, refrigerator, car/truck/tractor, cattle.
‡>75th percentile of the number of persons living in the household.
§Hinterland and environs.
¶Includes casual laborer, artisan, and others.
#By nitrite-positive urine dipstick test (Combur 10, Roche Diagnostics, Mannheim, Germany).

not attributable to recent antimalarial medication (7 persons with type 2 diabetes mellitus vs. 13 persons without type 2 diabetes mellitus; p = 0.32), and, notably, 74/524 (14.1%) of the patients with type 2 diabetes mellitus who took metformin-based drugs were infected compared with 34/131 (26.0%) of those who did not (p = 0.01). Among controls and patients with hypertension, the *P. falciparum* prevalence was similar (35/377, 9.3% for controls; 46/411, 11.2% for patients with hypertension; p = 0.38), and in each case, it was comparatively higher among patients with type 2 diabetes mellitus (p = 0.003 for controls; p = 0.03 for patients with hypertension).

Several factors that differed between persons with and those without diabetes mellitus (Table 1) were associated with *P. falciparum* infection (Table 2). However, age-adjusted multivariate analysis confirmed that the odds of *P. falciparum* infection in patients with type 2 diabetes mellitus were increased (aOR 1.46; Table 2). This risk increase was still discernible in the same model comparing patients with type 2 diabetes mellitus with controls (aOR 1.68, 95% CI 1.06–2.65; p = 0.027) or patients with hypertension (aOR 1.38, 95% CI 0.94–2.02; p = 0.096), or when separating into metropolitan area (aOR 1.67, 95% CI 1.12–2.48; p = 0.01) and other residence (aOR 1.32, 95% CI 0.76–2.29; p = 0.33).

According to the multivariate model, exchanging type 2 diabetes mellitus with glucose concentration showed that each mmol/L increase in blood glucose increased the risk for *P. falciparum* infection by 5% (aOR 1.05, 95% CI 1.02–1.09; p = 0.002). Among patients with type 2 diabetes mellitus, a stepwise approach identified 8.6 mmol/L glucose concentration as the significant threshold of risk increase (aOR 1.63, 95% CI 1.07–2.48; p = 0.02).

Conclusions

This study provides evidence for increased risk for *P. falciparum* infection in patients with type 2 diabetes mellitus (Table 2). Most infections were detected by PCR exclusively, and all were asymptomatic.

Submicroscopic and asymptomatic *P. falciparum* infections are common in areas where malaria is endemic. In adults, PCR may identify up to 50% of infections, although only a few infections are diagnosed by microscopy (*10*). These submicroscopic infections tend to increase in areas of low endemicity and with patient age (*10)*.

An increased risk for *P. falciparum* infection in persons with diabetes mellitus might become clinically relevant (and microscopically detectable) under several conditions. The impact of semi-immunity on controlling parasitemia may weaken with advancing type 2 diabetes mellitus and immune dysfunction (*5*), as suggested by the observed risk increase with increasing glucose concentration. Conversely, children who lack semi-immunity but have more severe type 1 diabetes mellitus may be particularly prone to malaria. Such vulnerability is also conceivable for women with gestational diabetes whose immune

Table 2. Univariate and multivariate associations with *Plasmodium falciparum* infection, Kumasi, Ghana, 2007–2008*

Parameter	Total no. patients	*P. falciparum* infection, no. (%)	Univariate analysis		Multivariate analysis	
			OR (95% CI)	p value	aOR (95% CI)	p value
Diabetes mellitus type 2						
No	791	81 (10.3)	1			
Yes	675	108 (16.0)	1.67 (1.22–2.27)	0.001	1.46 (1.06–2.03)	0.021
Gender						
F	1,113	124 (11.2)	1			
M	353	65 (18.5)	1.80 (1.29–2.50)	<0.0001	2.13 (1.50–3.03)	<0.0001
Wealth score						
≥25th percentile	923	94 (10.2)				
<25th percentile †	536	94 (17.6)	1.88 (1.38–2.56)	<0.0001	1.76 (1.27–2.42)	0.001
Literacy						
Able to read	947	103 (10.9)	1			
Unable to read	514	85 (16.6)	1.63 (1.20–2.23)	0.002	1.59 (1.11–2.28)	0.011
Formal education						
Any	1,091	126 (11.6)	1			
None	370	62 (16.8)	1.54 (1.11–2.15)	0.010		
Living condition						
Uncrowded	1,147	133 (11.6)	1			
Crowded‡	297	52 (17.5)	1.61 (1.14–2.29)	0.007		
Smoking						
Never	1,380	171 (12.4)	1			
Current or quit	84	18 (21.4)	1.92 (1.11–3.32)	0.019		
Ethnicity						
Akan	1,277	156 (12.3)	1			
Others	188	33 (17.6)	1.52 (1.01–2.30)	0.045		
Residence						
Kumasi metropolitan	1,079	121 (11.2)	1			
Kumasi outskirts	336	64 (19.2)	1.87 (1.34–2.61)	<0.0001		
Elsewhere §	48	4 (8.3)	0.72 (0.25–2.03)	0.533		
Occupation						
Public servant	238	17 (7.1)	1			
Trader	388	50 (12.9)	1.92 (1.08–3.42)	0.026		
Farmer	113	34 (30.6)	5.74 (3.04–10.86)	<0.0001		
Other¶	335	38 (11.3)	1.66 (0.92–3.02)	0.095		
Unemployed	386	49 (12.8)	1.90 (1.07–3.39)	0.029		

*OR, odds ratio; CI, confidence interval; aOR, adjusted odds ratio. Age and gender were a priori included in the multivariate model. Further variables for inclusion in the model were identified by factor analysis excluding multicollinear parameters (1: retained diabetes, excluded occupation; 2: retained literacy, excluded education, smoking; 3: retained wealth, excluded living condition, ethnicity). The same model results from a logistic regression analysis initially including all above listed parameters, and then removing in a stepwise backward fashion all factors not associated with *P. falciparum* infection in multivariate analysis (p > 0.05). Inserting any of the excluded variables back into the model did not change the aOR of patients with type 2 diabetes mellitus by >7% each, suggesting the absence of substantial confounding. Leaving all parameters in the model yielded an aOR for patients with type 2 diabetes mellitus of 1.36 (95% CI, 0.98–1.90; p = 0.07). Alternatively, propensity score adjustment of that analysis, i.e. reducing covariates into a single variable, produced aOR = 1.41 (95% CI, 1.02–1.95; p = 0.04).
†<25th percentile of a calculated index of 11 markers of wealth.
‡Crowded living condition, >75th percentile of the number of persons living in the household, i.e., n>8.
§Hinterland and environs.
¶Includes casual labourer, artisan, and others.

systems are relatively naive with regard to pregnancy-specific *P. falciparum* (*11*). Moreover, low-level infections in patients with type 2 diabetes mellitus may constitute an unrecognized infectious reservoir in areas where malaria is endemic (*10*). The lowered *P. falciparum* prevalence under metformin medication accords with the biguanides' antimalarial efficacy (*12*).

Our data stem from a study that was not designed to assess influences on *P. falciparum* infection in a heterogeneous population. Multivariate analysis cannot exclude unmeasured confounders, and association does not mean causality. As a limitation, factors influencing infection were not specifically identified during recruitment and thus were not included in analysis. Also, despite adjusting for proxy indicators, e.g., wealth, exposure to infection might still have differed between the study groups, considering the patchy malaria transmission in Kumasi (*6*). Nonetheless, increased odds of *P. falciparum* in patients with type 2 diabetes mellitus were found after stratification by subgroups or residence. Ultimate corroboration would need a prospective, longitudinal study controlling for exposure (possibly monitored by serologic markers of transmission).

Although the actual reasons for the increase of *P. falciparum* infection are unclear, the risk increase with rising glucose concentration is a sign of biologic plausibility. Such risk could result from impaired defense against liver and/or blood-stage parasites and from prolonged persistence. In type 2 diabetes mellitus, decreased T cell–mediated immunity but limited impact on humoral responses are discussed (*5*). Mechanistically, increased glucose availability may feed *P. falciparum* growth as seen in vitro (*13*). Also, patients with diabetes might receive more infectious mosquito bites: olfactory signals mediate mosquito attraction (*14*), and these, including expiration, are subtly altered in persons with type 2 diabetes mellitus (*15*).

The rapid proliferation of type 2 diabetes mellitus in sub-Saharan Africa may put an increasing number of persons at risk for *Plasmodium* infection and malaria. Thus, the magnitude of both diabetes mellitus and malaria in sub-Saharan Africa warrants further investigation into the relevance and causes of our finding

Acknowledgments

We thank all participants at Komfo Anokye Teaching Hospital and acknowledge the study team of the Kumasi Diabetes and Hypertension Study for on-site recruitment, data and sample collection, and laboratory analyses.

This study was supported by Charité Universitätsmedizin Berlin (grant 89539150) and HemoCue, Germany (photometers and consumables).

Ms Danquah is a nutrition scientist at the Institute of Tropical Medicine and International Health, Berlin. Her research interests include nutritional aspects in susceptibility to infectious diseases and in noncommunicable diseases in sub-Saharan Africa.

References

1. Lopez AD, Mathers CD, Ezzati M, Jamison DT, Murray CJ. Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. Lancet. 2006;367:1747–57. DOI: 10.1016/S0140-6736(06)68770-9
2. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for the year 2030. Diabetes Care. 2004;27:1047–53. DOI: 10.2337/diacare.27.5.1047
3. Brancati FL, Kao WHL, Folsom AR, Watson RL, Szklo M. Incident type 2 diabetes mellitus in African American and white adults: the Atherosclerosis Risk in Communities study. JAMA. 2000;283:2253–9. DOI: 10.1001/jama.283.17.2253
4. Amoah AG, Owusu SK, Adjei S. Diabetes in Ghana: a community based prevalence study in Greater Accra. Diabetes Res Clin Pract. 2002;56:197–205. DOI: 10.1016/S0168-8227(01)00374-6
5. Muller LM, Gorter KJ, Hak E, Goudzwaard WL, Schellevis FG, Hoepelman AI, et al. Increased risk of common infections in patients with type 1 and type 2 diabetes mellitus. Clin Infect Dis. 2005;41:281–8. DOI: 10.1086/431587
6. Afrane YA, Klinkenberg E, Drechsel P, Owusu-Daaku K, Garms R, Kruppa T. Does irrigated urban agriculture influence the transmission of malaria in the city of Kumasi, Ghana? Acta Trop. 2004;89:125–34. DOI: 10.1016/j.actatropica.2003.06.001
7. Snounou G, Viriyakosol S, Jarra W, Thaithong S, Brown KN. Identification of the four human malaria parasite species in field samples by the polymerase chain reaction and detection of a high prevalence of mixed infections. Mol Biochem Parasitol. 1993;58:283–92. DOI: 10.1016/0166-6851(93)90050-8
8. World Health Organization. Definition, diagnosis and classification of diabetes mellitus and its complications: report of a WHO consultation. Part 1: diagnosis and classification of diabetes mellitus. Geneva: The Organization; 1999
9. World Health Organization/International Society of Hypertension. Guidelines for the management of hypertension. J Hypertens. 1999;17:151–83.
10. Okell LC, Ghani AC, Lyons E, Drakeley CJ. Submicroscopic infection in *Plasmodium falciparum*–endemic populations: a systematic review and meta-analysis. J Infect Dis. 2009;200:1509–17. DOI: 10.1086/644781
11. Fried M, Nosten F, Brockman A, Brabin BJ, Duffy PE. Maternal antibodies block malaria. Nature. 1998;395:851–2. DOI: 10.1038/27570
12. Jones K, Ward SA. Biguanide-atovaquone synergy against *Plasmodium falciparum* in vitro. Antimicrob Agents Chemother. 2002;46:2700–3. DOI: 10.1128/AAC.46.8.2700-2703.2002
13. Jensen MD, Conley M, Helstowski LD. Culture of *Plasmodium falciparum*: the role of pH, glucose, and lactate. J Parasitol. 1983;69:1060–7. DOI: 10.2307/3280864
14. Takken W, Knols BG. Odor-mediated behavior of Afrotropical malaria mosquitoes. Annu Rev Entomol. 1999;44:131–57. DOI: 10.1146/annurev.ento.44.1.131
15. Dalton P, Gelperin A, Preti G. Volatile metabolic monitoring of glycemic status in diabetes using electronic olfaction. Diabetes Technol Ther. 2004;6:534–44. DOI: 10.1089/1520915041705992

Address for correspondence: Frank P. Mockenhaupt, Institute of Tropical Medicine and International Health Berlin, Charité–University Medicine Berlin, Spandauer Damm 130, 14050 Berlin, Germany; email: frank.mockenhaupt@charite.de

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 2009, p. 1753–1759 Vol. 53, No. 5
0066-4804/09/$08.00+0 doi:10.1128/AAC.01723-08

Reduced Efficacy of Intermittent Preventive Treatment of Malaria in Malnourished Children▽

Ina Danquah,[1] Ekkehart Dietz,[2] Philipp Zanger,[3] Klaus Reither,[4] Peter Ziniel,[5] Ulrich Bienzle,[1]† and Frank P. Mockenhaupt[1]*

Institute of Tropical Medicine and International Health, Charité University Medicine, Berlin, Germany[1]; Institute of Biometry and Clinical Epidemiology, Charité University Medicine, Berlin, Germany[2]; Institute of Tropical Medicine, University of Tuebingen, Tuebingen, Germany[3]; Department of Infectious Diseases and Tropical Medicine, University of Munich, Munich, Germany[4]; and Northern Region Malaria Project, Tamale, Ghana[5]

Received 30 December 2008/Returned for modification 25 January 2009/Accepted 7 February 2009

Intermittent preventive treatment in infants with sulfadoxine-pyrimethamine (IPTi-SP) reduces malaria episodes by 20 to 59% across Africa. This protective efficacy, however, may be affected by the high frequency of malnutrition in African infants. We analyzed the impact of malnutrition as defined by anthropometry on the incidence of malaria and on the protective efficacy of IPTi in a cohort of 1,200 children in northern Ghana, where malaria is hyperendemic. These children received IPTi-SP or placebo at 3, 9, and 15 months of age and were monitored until 24 months of age. Malnutrition was present in 32, 40, and 50% of children at ages 3, 9, and 15 months, respectively. It was associated with increased risks of severe anemia and death but not an increased risk of malaria. Although malaria slightly contributed to chronic malnutrition, IPTi did not substantially improve child growth. Importantly, the protective efficacies of IPTi in malnourished children were roughly half or even less of those observed in nonmalnourished children. In the first year of life, IPTi reduced the incidence of malaria to a significantly lesser extent in infants who received both doses in a malnourished condition (25%; 95% confidence interval [CI], −7 to 48%) compared to that of nonmalnourished children (46%; 95% CI, 30 to 58%; $P = 0.049$). Moreover, in contrast to nutritionally advantaged children, the rate of severe malaria appeared to be increased in malnourished children who took IPTi. IPTi might exhibit reduced efficacy in regions of abundant malnutrition. Concomitant nutrition programs may be needed in these places to achieve the desired impact.

Intermittent preventive treatment in infants (IPTi) with sulfadoxine-pyrimethamine (SP) appears to be a promising tool of malaria control in young children. The initial IPTi trial in Tanzania reported a protective efficacy (PE) against uncomplicated malaria in infancy of 59% and some degree of protection persisting into the second year of life (28, 29). In subsequent studies at sites of differing endemicity in sub-Saharan Africa, protection in infancy was confirmed; however, the PEs were lower, at 20 to 33% (5, 12, 15, 17, 23). We have reported previously that in Tamale, northern Ghana, IPTi reduced the incidence of asymptomatic parasitemia, uncomplicated malaria, and severe anemia from 3 to 24 months of age by 29, 17, and 15%, respectively. These effects were greatest in the first year of life and less pronounced in the second (23).

As in many regions of Africa, malnutrition is abundant in northern Ghana, reaching prevalences as high as 50% in preschool children, depending on seasonality and food availability (32 and http://www.who.int/nutgrowthdb/database/countries/nchs_reference/gha.pdf). Malnutrition causes relative immunosuppression, and repeated or chronic infections may contribute to poor nutritional status (27). However, the effect of malnutrition on malaria is less clear cut than would be expected: protein-energy malnutrition has been associated with greater malaria morbidity and mortality in some areas but not in others (4, 6, 8, 21, 24, 30). Moreover, the risk of antimalarial treatment failure appears to be increased in malnourished children (13, 14, 22, 39). Taken together, these findings suggest that malnutrition is one factor contributing to malaria-associated morbidity and that malaria control strategies without concomitant nutrition programs may not have the desired impact on childhood morbidity on a large scale (8). We hypothesized that malnutrition affects both malaria morbidity and IPTi efficacy. Alternatively, IPTi might improve children's growth and nutritional status. We reanalyzed data from a cohort from northern Ghana (23) regarding the effect of malnutrition on the PE of IPTi, and here we report the results of this secondary analysis.

MATERIALS AND METHODS

Study site and IPTi trial. The IPTi trial was conducted between March 2003 and July 2005 at Bulpeila Health Centre, located in a semiurban outskirt of Tamale, northern Ghana. Despite a population of 350,000, the town is of rural character and spread over a vast area. Subsistence farming and small-scale trade are the main income sources. Climate and vegetation are of the savanna type, with rains from May to October. Malaria in the region is hyperendemic, with modest seasonal variation; underweight and stunting each affect approximately one out of four children (8). At the time the study was carried out, bed net usage was low (3%), and malaria control basically consisted of chloroquine treatment, which achieved cure rates of <50% (22).

The study was a randomized, double-blind, placebo-controlled trial on the efficacy of SP given alongside the Expanded Program on Immunization (EPI) (http://clinicaltrials.gov; NCT00168948). Informed written consent was obtained

* Corresponding author. Mailing address: Institute of Tropical Medicine and International Health, Spandauer Damm 130, 14050 Berlin, Germany. Phone: 49 30 30116 815. Fax: 49 30 30116 888. E-mail: frank.mockenhaupt@charite.de.
† Deceased.
▽ Published ahead of print on 17 February 2009.

TABLE 1. Prevalence of malnutrition at application of intermittent preventive treatment at 3, 9, and 15 months of age[a]

Dose (*n*)	No. (%) of children who were:				
	Nonmalnourished	Malnourished	Underweight	Wasting	Stunting
1 (1,199)	809 (67.5)	388 (32.4)	84 (7.0)	292 (24.4)	102 (8.5)
2 (1,127)	672 (59.6)	451 (40.0)	268 (23.8)	290 (25.7)	182 (16.1)
3 (1,086)	538 (49.5)	540 (49.7)	367 (33.8)	218 (20.1)	411 (37.8)

[a] Two children at dose 1, four children at dose 2, and eight children at dose 3 could not be categorized as malnourished or nonmalnourished due to at least one missing anthropometrical measure. The calculated mean S/P dosage ratios at IPTi doses 1, 2, and 3 in malnourished children were 52.1 ± 12.9/2.6 ± 0.7, 39.1 ± 5.0/2.0 ± 0.3, and 33.4 ± 3.7/1.7 ± 0.2 mg/kg, respectively. The respective figures for nonmalnourished children were 43.5 ± 5.5/2.2 ± 0.3, 32.5 ± 3.1/1.6 ± 0.2, and 28.5 ± 2.5/1.4 ± 0.1 mg/kg, respectively ($P < 0.0001$ by Mann Whitney U tests for all comparisons).

from the participants' parent(s). The study protocol was approved by the Ethics Committee, University for Development Studies, Tamale, Ghana. Details have been described elsewhere (23). In brief, a total of 1,200 children received half a tablet of SP (dosage ratio, 125 mg S per kg of body weight/6.25 mg P per kg of body weight; Fansidar; Roche, Basel, Switzerland) or placebo at 3, 9, and 15 months of age and were monitored at 6, 12, 18, 21, and 24 months of age. For passive case detection, parents were instructed to bring their children to the health center in case of any health problem. A civil conflict involving changing curfews (until August 2004) occasionally impeded children from attending the health center or hospital in the late afternoon. At scheduled visits, children were clinically examined, a medical history was obtained, and a venous blood sample was collected. Blood samples also were collected at unscheduled visits in case of fever (axillary temperature, ≥37.5°C), a history of fever, or when requested by the clinician. Asexual malaria parasites were counted against 500 white blood cells on Giemsa-stained thick blood films, and hemoglobin (Hb) was measured (HemoCue, Ångelholm, Sweden). Malaria was defined as parasitemia of any density plus fever or a voluntarily reported history of fever within the preceding 48 h, severe anemia was defined as an Hb level of <7 g/dl (31), and severe malaria was defined according to WHO criteria (37). Malaria was treated under observation with artesunate (Plasmotrim; Mepha, Switzerland) at a dose of 4 mg/kg (with a double dose on first day) for 5 days. Other diseases were treated according to Ghana Health Service guidelines (10).

Measures of malnutrition. At each regular visit, weight and length/height were measured and related to age and sex. Malnutrition was defined by these anthropometric parameters according to the WHO 2006 standard reference data (WHO Anthro software, version 2.0.2.) (38). A weight-for-age z-score (waz) of ≤2 standard deviations (SD) characterized underweight, indicating general malnutrition. Likewise, for weight-for-height (whz) and height-for-age z-scores (haz), ≤2 SD denoted wasting (acute malnutrition) and stunting (chronic malnutrition), respectively. In this paper, the term overall malnutrition refers to children in at least one of these conditions. For the present analysis, the nutritional status was categorized using a static and a dynamic approach. Statically, the nutritional state was determined at baseline, at 1 year of age, and at each IPTi administration. Alternatively, and to account for the dynamic nature of the nutritional status, a nonparametric mixture model for longitudinal data (1) clustered the individual follow-up curves of anthropometrical z-scores from 3 to 24 months of age. Children whose scores belonged to a cluster of ≤2 SD were categorized as underweight, wasted, or stunted.

Data management and statistical analysis. In a first step, the effect of the nutritional status per se (static definition) on the main outcomes (asymptomatic parasitemia, uncomplicated malaria, severe malaria, severe anemia, and death) was analyzed by comparing incidence densities (ID; i.e., events per person years at risk [PYAR]) between malnourished and nonmalnourished children using negative binomial regression. Following an event, a child was considered not to be at the respective risk for 3 weeks, and the person time was reduced accordingly. Incidence rate ratios (IRRs; $ID_{nonmalnourished}/ID_{malnourished}$) were adjusted for the intervention group. Vice versa, the impact of disease on the development of anthropometrical z-scores from 3 to 24 months of age was assessed by general estimating equation (GEE) accounting for repeated, correlated observations. The results are given as the means of differences in z-scores (Δ z-score) between children with at least one event and those with none, adjusted for the intervention. To further evaluate the effect of IPTi on anthropometrical development, time-dependent changes in weight, length, mid-upper arm circumference (MUAC), and z-scores were calculated, compared by Mann-Whitney U tests, and expressed as differences per month.

As the main analysis, we estimated the PEs of IPTi in strata of nutritional status. For that, the IDs of asymptomatic parasitemia, uncomplicated malaria, severe malaria, severe anemia, and death were calculated. As before, the person time was reduced for 3 weeks following an event. To account for repeated, dependent measures, the efficacies of IPTi ($1 - ID_{SP}/ID_{placebo}$), 95% confidence intervals (CI), and *P* values were calculated by negative binomial regression and adjusted for the rainy season (http://www.fao.org/nr/water/aquastat/main/index.stm) and food availability (2 and http://documents.wfp.org/stellent/groups/public/documents/ena/wfp036439.htm). PEs of IPTi in malnourished and nonmalnourished children were estimated for various strata and observation periods. For the static classification of nutritional status, these were (i) from 3 to 24 months of age, grouping children into nonmalnourished ($n = 809$) and malnourished ($n = 388$) based on recruitment anthropometry; (ii) from 3 to 12 months of age, for which children were grouped according to whether they received both IPTi doses in a nonmalnourished ($n = 527$) or malnourished condition ($n = 210$); (iii) from >12 to 24 months of age, with the status at IPTi dose 3 defining the nutritional situation (nonmalnourished, $n = 538$; malnourished, $n = 540$); and (iv) each 6 months following an IPTi dose. The significance of differences in the PEs between nutritional strata was assessed by Wald tests. Calculations of PEs were repeated for the period of 3 to 24 months, applying nutritional strata derived from the nonparametric mixture model for longitudinal data (nonmalnourished, $n = 200$; malnourished, $n = 999$).

The software packages SPSS 14.0 (SPSS Inc., Chicago, IL) and STATA 9.0 (StataCorp LP, Chicago, IL) were used.

RESULTS

Study population. Between March and September 2003, 1,200 infants were randomly assigned to receive SP or placebo. Baseline characteristics, drop-out rates, and mortality rates were similar in both study arms (23). Eighty-nine percent of the children received all three doses of IPTi, and 87% completed the follow-up until 24 months of age. The prevalences of overall malnutrition at 3, 9, and 15 months of age were 32, 40, and 50%, respectively (Table 1). Baseline characteristics of malnourished and nonmalnourished children were similar (data not shown).

Interaction between nutritional status and malaria. We assessed the incidences of asymptomatic parasitemia, uncomplicated malaria, severe anemia, and death during the complete follow-up period. These data were compared between children with and without malnutrition (including stunting, wasting, and being underweight) at recruitment. The nutritional status had no influence on asymptomatic parasitemia or on uncomplicated malaria; e.g., the latter occurred at a rate of 1.17/year and 1.11/year in malnourished and nonmalnourished children, respectively ($P = 0.38$). Two significant associations were observed: malnutrition increased the risk of dying by 89% (IRR, 1.89; 95% CI, 1.03 to 3.47; $P = 0.04$), and stunting increased the risk of severe anemia by 49% (IRR, 1.49; 95% CI, 1.07 to 2.07; $P = 0.02$).

The impact of the disease status on the development of nutritional indices was examined by GEE. Parasitemia and malaria were associated with measures indicating stunting: haz

TABLE 2. Child growth according to age and use of intermittent preventive treatment

Treatment status and observation period	Mean wt gain (g/mo)	Mean ht gain (mm/mo)	Mean MUAC gain (mm/mo)	Mean change in z-scores/mo		
				wt for age	wt for ht	ht for age
First year of life						
Placebo	235.6	133.6	−0.9	−0.2	−0.1	−0.2
SP	249.7	133.3	0	−0.2	−0.1	−0.2
Δ (SP−placebo)[a]	14.1[b]	−0.3	0.9	0	0	0
Second year of life						
Placebo	190.1	73.2	4.4	<0.1	0.1	−0.1
SP	191	75.4	4.8	<0.1	0.1	−0.1
Δ (SP−placebo)[a]	0.9	2.1	0.4	0	0	0
3–24 mo						
Placebo	210.3	9.8	0.2	−0.1	<0.1	−0.1
SP	215.3	9.9	0.3	−0.1	<0.1	−0.1
Δ (SP−placebo)[a]	5	0.1	0.1	0	0	0

[a] Δ (SP−placebo) means the mean growth in the SP group minus the mean growth in the placebo group.
[b] $P < 0.05$ by Mann-Whitney U tests.

scores were aggravated in children who experienced at least one episode compared to those of unaffected children (Δ haz for parasitemia, −0.17 [$P = 0.02$]; Δ haz for malaria, −0.18 [$P = 0.01$]). Regarding severe anemia, this influence was more pronounced (Δ haz, −0.30; $P < 0.0001$) and also was discernible in terms of being underweight (Δ waz, −0.21; $P = 0.001$).

Influence of IPTi on child growth. The changes in weight, height, MUAC, and z-scores with increasing age are displayed in Table 2. IPTi improved weight gain exclusively in the first year of life and did so only to a small extent (14 g/month; $P = 0.02$). Moreover, this effect was restricted to children who were not malnourished when receiving IPTi doses 1 and 2 (nonmalnourished, 19 g/month [$P = 0.08$]; malnourished, 0.3 g/month [$P = 0.73$]). No beneficial impact of IPTi on nutritional indices was observed in the second year of life or for the complete follow-up period (Table 2).

Efficacy of IPTi in malnourished children. Table 3 shows the PEs of IPTi against the main outcome parameters according to nutritional status and adjusted for the rainy season and food availability. Overall, the PEs of IPTi in malnourished children were roughly half or even less of those observed in nonmalnourished children. Also, in this analysis, significant risk reductions due to IPTi were observed only among nonmalnourished children. For instance, IPTi reduced the number of episodes of uncomplicated malaria by only 9.5% ($P = 0.32$) in malnourished children but by 18.4% ($P = 0.006$) among their nonmalnourished counterparts (Table 3). Generally, these differences in the impact of IPTi according to nutritional status did not yield statistical significance. Nonetheless, during infancy IPTi reduced malaria incidence to a significantly greater extent in nutritionally advantaged participants (46%) than in malnourished children (25%; $P = 0.049$). This was particularly pronounced following IPTi dose 1 (34% for nonmalnourished children, −14% for malnourished children; $P = 0.02$) (Table 4). A similar difference was observed with respect to severe anemia following IPTi dose 2 ($P = 0.002$) (Table 4) and in the second year of life ($P = 0.047$) (Table 3). The comparatively diminished efficacies of IPTi in malnourished children largely were confirmed not only for periods of 6 months following each treatment dose (Table 4) but also by a nonparametric mixture model (1) that categorizes nutritional status during the complete follow-up period (Fig. 1). However, in children who experienced malaria episodes during the 6 months after an IPTI administration, time periods until the respective first or only event were only slightly reduced in malnourished compared to those in nonmalnourished children and only following dose 2 (malnourished, 11.3 ± 8.5 weeks; nonmalnourished, 12.1 ± 8.6 weeks) and dose 3 (malnourished, 10.7 ± 6.8 weeks;

TABLE 3. PEs of intermittent preventive treatment separated by nutritional status[a]

Observation period	Protective efficacies (95% CI) of IPTi					
	Asymptomatic parasitemia		Uncomplicated malaria		Severe anemia	
	Nonmalnourished	Malnourished	Nonmalnourished	Malnourished	Nonmalnourished	Malnourished
First year of life	52.8 (32.1–67.2)[b]	16.0 (−35.4–47.9)	46.0 (30.0–58.4)[b,e]	25.2 (−7.3–47.9)	48.2 (16.3–68.0)[c]	−31.8 (−168.9–35.4)
Second year of life	26.6 (5.0–43.3)[d]	14.6 (−10.3–33.9)	18.6 (1.8–32.5)[d]	−4.5 (−26.1–13.4)	21.3 (−8.3–42.8)[e]	10.2 (−17.3–31.2)
3–24 mo	24.7 (12.5–35.3)[b]	10.9 (−9.5–27.5)	18.4 (5.7–29.4)[c]	9.5 (−10.3–25.7)	22.7 (3.0–38.5)[d]	16.8 (−15.6–40.1)

[a] Data are PEs [1 − ($\text{Events}_{\text{nonmalnourished}}/\text{PYAR}_{\text{nonmalnourished}}$)/($\text{Events}_{\text{malnourished}}/\text{PYAR}_{\text{malnourished}}$)] and 95% CIs calculated by negative binomial regression adjusted for rainfall and food availability at the respective IPTi dose administration(s). For the definition of nutritional strata, see Materials and Methods. In the first year of life, 420 children received IPTi dose 1 or 2 in a malnourished condition. In these children, PEs (95% CIs) were the following: asymptomatic parasitemia, 37.1 (11.7 to 55.2); malaria, 14.4 (−15.2 to 36.4); and severe anemia, 17.5 (−56.1 to 56.4).
[b] $P < 0.0001$.
[c] $P < 0.01$.
[d] $P < 0.05$.
[e] PE differs significantly between malnourished and nonmalnourished children ($P < 0.05$ by Wald test).

TABLE 4. PEs of intermittent preventive treatment during 6 months following each dose stratified for nutritional status at time of dose administration[a]

Observation period	Protective efficacies (95% CI) of IPTi					
	Asymptomatic parasitemia		Uncomplicated malaria		Severe anemia	
	Nonmalnourished	Malnourished	Nonmalnourished	Malnourished	Nonmalnourished	Malnourished
6 mo after dose 1	52.0 (32.3–65.9)[b]	36.1 (−2.4–60.1)	33.8 (14.6–48.7)[c,e]	−13.6 (−63.2–20.9)	38.9 (−18.4–68.5)	−13.7 (−174.5–52.9)
6 mo after dose 2	43.1 (14.3–62.3)[c]	25.8 (−14.2–51.8)	36.5 (16.8–51.6)[c]	35.3 (10.3–53.0)[c]	54.6 (26.7–71.9)[c,e]	−39.1 (−138.3–18.9)
6 mo after dose 3	15.1 (−15.7–37.8)	2.5 (−32.0–28.0)	22.9 (3.9–38.1)[d]	−1.2 (−26.0–18.8)	11.6 (−30.8–40.2)	19.1 (−10.9–41.0)

[a] Data are PEs [1 − (Events$_{nonmalnourished}$/PYAR$_{nonmalnourished}$)/(Events$_{malnourished}$/PYAR$_{malnourished}$)] and 95% CIs calculated by negative binomial regression adjusted for rainfall and food availability at the respective IPTi dose administration(s).
[b] $P < 0.0001$.
[c] $P < 0.01$.
[d] $P < 0.05$.
[e] PE differs significantly between malnourished and nonmalnourished children ($P < 0.05$ by Wald test).

nonmalnourished, 11.1 ± 6.8 weeks) but not so following dose 1 (malnourished, 14.1 ± 5.0 weeks; nonmalnourished, 13.3 ± 6.2 weeks) (for all comparisons, $P > 0.5$ by Mann-Whitney U tests).

A specific constellation was observed regarding IPTi, nutritional status, and severe malaria. Severe malaria occurred at similar rates in malnourished and nonmalnourished children (0.03/year for both groups). In the latter group, IPTi provided a nonsignificant degree of protection (PE, 14%; $P = 0.67$). Remarkably, however, the mere opposite was seen in children who were malnourished at recruitment: 14 episodes of severe malaria (0.046/year) occurred in children receiving SP, while only 5 episodes (0.016/year) occurred in the placebo group (PE, −169%; 95% CI, −670 to 6.3%; $P = 0.07$). This reversed impact of IPTi in malnourished children was significantly different from the protective effect in their nonmalnourished counterparts ($P = 0.04$). By repeating this analysis by applying the nonparametric mixture model as described above (1), the increased risk of severe malaria in malnourished children receiving SP appeared to be less pronounced but still was discernible (PE, −35%; 95% CI, −152 to 28%; $P = 0.35$; $P = 0.16$ by Wald test).

DISCUSSION

IPTi is a simple means of malaria control in young children, particularly when administered alongside the well-established EPI schedule. This approach has the potential to overcome one of the predominant problems in disease control in sub-Saharan Africa, i.e., limited access to health structures providing accurate treatment or prevention. Due to the advantages of low cost, single-dose treatment, and a favorable safety profile, most IPTi trials so far have used SP (5, 12, 15, 17, 23, 28), although the use of other drugs also is conceivable. The mode of action of IPTi with SP is not fully understood, but it comprises properties of both treatment and chemoprophylaxis, the latter appearing to predominate (23). The protective effect also may result from an attenuation or containment of parasites exposed to SP, consequently preventing clinical disease but providing an enhanced opportunity to develop protective immunity (11, 29). If that is true, some degree of SP resistance might be tolerable, but its exact level is unknown. In fact, SP-resistant *Plasmodium falciparum* parasites have spread throughout Africa in recent years, a development that also is discernible in Ghana (18, 20).

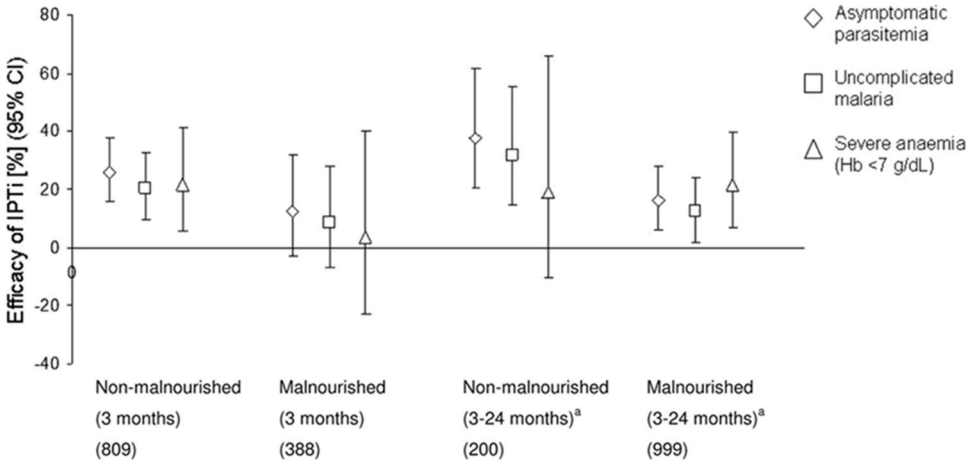

FIG. 1. PEs and 95% CIs of intermittent preventive treatment from 3 to 24 months of age by nutritional status. Efficacies of IPTi were obtained by negative binomial regression and were adjusted for rainfall and food availability. Adjustment for possible socioeconomic confounders and differences in clinical parameters at baseline did not lead to meaningful differences. Footnote a indicates that a nonparametric mixture model for longitudinal data modified according to Aitkin (1) classified the individual follow-up curves of anthropometrical z-scores from 3 to 24 months of age.

Malnutrition among infants is shamefully frequent in many parts of Africa and has been shown to affect antimalarial treatment responses, drug absorption, and immune responses, among others (13, 14, 22, 27, 33, 35, 39). Thus, it is appropriate to hypothesize that nutritional status influences the effect of IPTi. In fact, in the present study, the PEs of IPTi were roughly halved in malnourished children, and as regards malaria, this was significant in the first year of life, when this intervention usually achieves its highest impact (15, 23). Not astonishingly, therefore, IPTi did not improve the growth of children who were malnourished.

A general problem in prospectively relating the nutritional status to morbidity or IPTi efficacy is the dynamic nature of the former. Not only did the prevalence of malnutrition increase during follow-up but anthropometric measures also exhibited various longitudinal patterns, e.g., poor or good initial values that subsequently declined, persisted, or increased. Our post hoc analysis is simplified in that these dynamic changes largely are not accounted for. We nevertheless consider our results valid, because stratified analyses of shorter time periods following various starting points produced basically the same results (Table 4). We consider the static classification of nutrition comprehensible and tangible, and, in terms of potential nutritional intervention, the status of a child beginning with IPTi appears to be practically relevant. Reassuringly, the nonparametric mixture model (1), which categorized nutritional status during the complete follow-up period, essentially confirmed the findings produced by the static approach. Nevertheless, this limitation should be kept in mind when construing our data.

How could malnutrition abate the PE of IPTi? An increased incidence of malaria in malnourished infants could lead to the impression that IPTi is less effective in these children. However, such increased morbidity was not observed in the present study, contrasting with previous findings (4, 6, 30) but corresponding with others (24). Malnutrition did increase all-cause mortality. Due to the civil conflict during the study, many deaths occurred at home, and a reliable diagnosis was not available for all children. Most deaths, however, presumably resulted from gastroenteritis, respiratory infections, and malaria. In accordance with current concepts (27, 30), this indicates that malnutrition compromised antipathogen immunity. Considering the hypothesis that (possibly enhanced) immune responses to parasites attenuated or contained by SP treatment are part of the mode of action of IPTi (11, 29), immune suppression caused by poor nutritional status may translate into a reduced PE. Such could be reflected by efficacy differences between malnourished and nonmalnourished children increasing with age. However, in the present study this was not observed. Also, malnutrition may influence the efficacy of SP by reducing the contribution of immunity to parasite clearance (9). In fact, in malnourished children the risk of the failure of antimalarial treatment (16, 24), including SP (13, 39), appears to be increased, although not uniformly so (16, 19). Altered pharmacokinetics of SP in malnourished children also could play a role by causing, e.g., increased clearance, reduced drug concentrations, and reduced half-life, as has been shown for quinine (33). Likewise, oral chloroquine treatment has been reported to be associated with reduced peak and overall drug concentrations in children with kwashiorkor, suggesting decreased bioavailability (35). If the pharmacokinetic properties of SP were altered in malnourished children (and related to the reduced efficacy of IPTi), such influence would be expected to be pronounced. This is because undernourished infants in the present study received a fixed and, thus, comparatively higher dose of SP than well-nourished children. Regrettably, no pharmacokinetic data of SP in malnourished children, let alone in IPTi per se, are available. This gap urgently needs to be closed, and the selection of alternative or future drugs for IPTi also should allow for pharmacokinetic properties.

What is the relevance of a reduced efficacy of IPTi in malnourished children? Affirmatively, the potential of IPTi appears to be higher than thought (23), considering the 46% PE against malaria in well-nourished children during the first year of life. Contrarily, the abundance of malnutrition in African infants may impair the value of one of the few available malaria control measures. The worsening international food crisis primarily affects the poor and vulnerable, and malnutrition in African children consequently can be expected to increase. Already, the average Ghanaian family spends some 70% of its budget on food (http://www.irinnews.org/Report.aspx?ReportId=78389). At present, it is unknown whether, and after which time period, malnourished children would benefit from refeeding in terms of IPTi. Moreover, individual nutritional assessments preceding IPTi would question the concept of a simple and affordable tool piggybacked onto the EPI system. Such operational difficulties are key issues in implementing IPTi and, overall, in reducing morbidity and mortality among African children. However, as stated previously (8) and confirmed here, malaria control programs will have limited effects without targeting the underlying causes, including malnutrition. On a large scale, training to detect poor nutritional status, nutritional counseling, and the education of caretakers and feeding programs are needed. Specifically, operational trials assessing the potential impact of refeeding on the efficacy of IPTi are warranted.

In malnourished children, the protective effect of IPTi against severe malaria appeared to be reversed, i.e., malnourished children receiving SP experienced an excess of episodes. Because of small numbers and borderline statistical significance, this finding needs to be interpreted with caution. As reported previously (23), most of these cases emerged during 16 to 24 months of age and were due to severe malarial anemia (Hb $<$ 5 g/dl). Folate deficiency complicating overall malnutrition could partially explain our findings: in the 1970s, folate deficiency was seen in 70% of young Ghanaian children (25), and no major improvement was observed recently in neighboring Togo (3). Deficient children will experience a decrease in plasma tetrahydrofolate concentrations after 3 to 4 months of insufficient folate intake, which will result in increasing megaloblastic anemia (36). SP inhibits the *P. falciparum* dihydropteroate synthase and dihydrofolate reductase, causing a disturbed folate metabolism in the parasite (26). In Wistar rats, SP induced folate deficiency (34), probably by the parasite's ability to assimilate external folate reservoirs, as observed in Malawian children (7). The administration of SP in malnourished and concomitantly folate-deficient children eventually may contribute to the development of severe anemia. Unfor-

tunately, concentrations could not be assessed in the present study. Further investigations are needed to rule out the above hypothesis.

In conclusion, in northern Ghana, IPTi in malnourished children achieved only roughly half the PE attainable under normal nutritional conditions. Moreover, malnourished children did not benefit from IPTi in terms of weight gain or growth and, possibly, bear the risk of rare but severe adverse events. This latter aspect should be looked at carefully in ongoing IPTi trials and during potential implementation. Further investigations of the interaction between malnutrition and IPTi and of the potential impact of nutritional programs in this regard are warranted. In regions where malnutrition is frequent, IPTi might not achieve its maximum effect.

ACKNOWLEDGMENTS

This work was supported by the German Ministry of Education and Research (grant 01KA0202), the German Academic Exchange Service (DAAD), and Charité University Medicine Berlin (grant 2005-543).

We thank all of the children and their families for participation in this study as well as all members of the Northern Region Malaria Project (NORMAP). We thank Andrew Seidu-Korkor and Elias Sory, Regional Health Administration, Tamale, Ghana, for general and infrastructural support.

We do not have a commercial or other association that might pose a conflict of interest.

REFERENCES

1. **Aitkin, M.** 1999. A general maximum likelihood analysis of variance components in generalized linear models. Biometrics **55:**117–128.
2. **Alderman, H., and G. Shively.** 1996. Economic reform and food prices: evidence from markets in Ghana. World Dev. **24:**521–534.
3. **Amouzou, E. K., N. W. Chabi, C. E. Adjalla, R. M. Rodriguez-Guéant, F. Feillet, C. Villaume, A. Sanni, and J. L. Guéant.** 2004. High prevalence of hyperhomocysteinemia related to folate deficiency and the 677CàT mutation of the gene encoding methylenetetrahydrofolate reductase in coastal West Africa. Am. J. Clin. Nutr. **79:**619–624.
4. **Caulfield, L. E., S. A. Richard, and R. E. Black.** 2004. Undernutrition as an underlying cause of malaria morbidity and mortality in children less than five years old. Am. J. Trop. Med. Hyg. **71:**55–63.
5. **Chandramohan, D., S. Owusu-Agyei, I. Carneiro, T. Awine, K. Amponsa-Achiano, N. Mensah, S. Jaffar, R. Baiden, A. Hodgson, F. Binka, and B. Greenwood.** 2005. Cluster randomised trial of intermittent preventive treatment for malaria in infants in area of high, seasonal transmission in Ghana. BMJ **331:**727–733.
6. **Deen, J. L., G. E. Walraven, and L. von Seidlein.** 2002. Increased risk for malaria in chronically malnourished children under 5 years of age in rural Gambia. J. Trop. Pediatr. **48:**78–83.
7. **Dzinjalamala, F. K., A. Macheso, J. G. Kublin, T. E. Taylor, K. I. Barnes, M. E. Molyneux, C. V. Plowe, and P. J. Smith.** 2005. Blood folate concentrations and in vivo sulfadoxine-pyrimethamine failure in Malawian children with uncomplicated *Plasmodium falciparum* malaria. Am. J. Trop. Med. Hyg. **72:**267–272.
8. **Ehrhardt, S., G. D. Burchard, C. Mantel, J. P. Cramer, S. Kaiser, M. Kubo, R. Otchwemah, U. Bienzle, and F. P. Mockenhaupt.** 2006. Malaria, anemia, and malnutrition in African children—defining intervention priorities. J. Infect. Dis. **194:**108–114.
9. **Francis, D., S. L. Nsobya, A. Talisuna, A. Yeka, M. R. Kamya, R. Machekano, C. Dokomajilar, P. J. Rosenthal, and G. Dorsey.** 2006. Geographic differences in antimalarial drug efficacy in Uganda are explained by differences in endemicity and not by known molecular markers of drug resistance. J. Infect. Dis. **193:**978–986.
10. **Ghana Health Service.** 2004. Standard treatment guidelines, 5th ed. Ghana National Drugs Programme, Accra, Ghana.
11. **Greenwood, B. M.** 2007. Intermittent preventive treatment of malaria in infants. Clin. Infect. Dis. **45:**26–28.
12. **Grobusch, M. P., B. Lell, N. G. Schwarz, J. Gabor, J. Dörnemann, M. Potschke, S. Oyakhirome, G. C. Kiessling, M. Necek, M. U. Langin, P. Klein Klouwenberg, A. Klopfer, B. Naumann, H. Altun, S. T. Agnandji, J. Goesch, M. Decker, C. L. Salazar, C. Supan, D. U. Kombila, L. Borchert, K. B. Koster, P. Pongratz, A. A. Adegnika, I. Glasenapp, S. Issifou, and P. G. Kremsner.** 2007. Intermittent preventive treatment against malaria in infants in Gabon—a randomized, double-blind, placebo-controlled trial. J. Infect. Dis. **196:**1595–1602.
13. **Hamel, M. J., T. Holtz, C. Mkandala, N. Kaimila, N. Chizani, P. Bloland, J. Kublin, P. Kazembe, and R. Steketee.** 2005. Efficacy of trimethoprim-sulfamethoxazole compared with sulfadoxine-pyrimethamine plus erythromycin for the treatment of uncomplicated malaria in children with integrated management of childhood illness dual classifications of malaria and pneumonia. Am. J. Trop. Med. Hyg. **73:**609–615.
14. **Hess, F. I., E. Nukuro, L. Judson, J. Rodgers, H. D. Nothdurft, and K. H. Rieckmann.** 1997. Anti-malarial drug resistance, malnutrition and socio-economic status. Trop. Med. Int. Health **2:**721–728.
15. **Kobbe, R., C. Kreuzberg, S. Adjei, B. Thompson, I. Langefeld, P. A. Thompson, H. H. Abruquah, B. Kreuels, M. Ayim, W. Busch, F. Marks, K. Amoah, E. Opoku, C. G. Meyer, O. Adjei, and J. May.** 2007. A randomized controlled trial of intermittent preventive antimalarial treatment in infants. Clin. Infect. Dis. **45:**16–25.
16. **MacArthur, J., G. M. Stennies, A. Macheso, M. S. Kolczak, M. D. Green, D. Ali, L. M. Barat, P. N. Kazembe, and T. K. Ruebush, Jr.** 2001. Efficacy of mefloquine and sulfadoxine-pyrimethamine for the treatment of uncomplicated *Plasmodium falciparum* infection in Machinga District, Malawi, 1998. Am. J. Trop. Med. Hyg. **65:**679–684.
17. **Macete, E., P. Aide, J. J. Aponte, S. Sanz, I. Mandomando, M. Espasa, B. Sigauque, C. Dobano, S. Mabunda, M. Dgedge, P. Alonso, and C. Menendez.** 2006. Intermittent preventive treatment for malaria control administered at the time of routine vaccinations in mozambican infants: a randomized, placebo-controlled trial. J. Infect. Dis. **194:**276–285.
18. **Maïga, O., A. A. Djimdé, V. Hubert, E. Renard, A. Aubouy, F. Kironde, B. Nsimba, K. Koram, O. K. Doumbo, J. Le Bras, and J. Clain.** 2007. A shared Asian origin of the triple-mutant dhfr allele in Plasmodium falciparum from sites across Africa. J. Infect. Dis. **196:**165–172.
19. **Méndez, F., A. Munoz, G. Carrasquilla, D. Jurado, M. Arévalo-Herrera, J. F. Cortese, and C. V. Plowe.** 2002. Determinants of treatment response to sulfadoxine-pyrimethamine and subsequent transmission potential in falciparum malaria. Am. J. Epidemiol. **156:**230–238.
20. **Mockenhaupt, F. P., G. Bedu-Addo, T. A. Eggelte, L. Hommerich, V. Holmberg, C. von Oertzen, and U. Bienzle.** 2008. Rapid increase in the prevalence of sulfadoxine-pyrimethamine resistance among Plasmodium falciparum isolated from pregnant women in Ghana. J. Infect. Dis. **198:**1545–1549.
21. **Mockenhaupt, F. P., S. Ehrhardt, J. Burkhardt, S. Y. Bosomtwe, S. Laryea, S. D. Anemana, R. N. Otchwemah, J. P. Cramer, E. Dietz, S. Gellert, and U. Bienzle.** 2004. Manifestation and outcome of severe malaria in children in northern Ghana. Am. J. Trop. Med. Hyg. **71:**167–172.
22. **Mockenhaupt, F. P., S. Ehrhardt, T. A. Eggelte, P. Agana-Nsiire, K. Stollberg, A. Mathieu, M. Markert, R. N. Otchwemah, and U. Bienzle.** 2005. Chloroquine-treatment failure in northern Ghana: roles of *pfcrt* T76 and *pfmdr1* Y86. Ann. Trop. Med. Parasitol. **99:**723–732.
23. **Mockenhaupt, F. P., K. Reither, P. Zanger, F. Roepcke, I. Danquah, E. Saad, P. Ziniel, S. Y. Dzisi, M. Frempong, P. Agana-Nsiire, F. Amoo-Sakyi, R. Otchwemah, J. P. Cramer, S. D. Anemana, E. Dietz, and U. Bienzle.** 2007. Intermittent preventive treatment in infants as a means of malaria control: a randomized, double-blind, placebo-controlled trial in northern Ghana. Antimicrob. Agents Chemother. **51:**3273–3281.
24. **Müller, O., M. Garenne, B. Kouyate, and H. Becher.** 2003. The association between protein-energy malnutrition, malaria morbidity and all-cause mortality in West African children. Trop. Med. Int. Health **8:**507–511.
25. **Neumann, C. G., M. E. Swenseid, M. Jacob, E. R. Stiem, and O. V. Dirige.** 1979. Biochemical evidence of thiamin deficiency in young Ghanaian children. Am. J. Clin. Nutr. **32:**99–104.
26. **Peters, W.** 1997. Chemotherapy and drug resistance in malaria. Academic Press, London, United Kingdom.
27. **Schaible, U. E., and S. H. Kaufmann.** 2007. Malnutrition and infection: complex mechanisms and global impacts. PLoS Med. **4:**e115.
28. **Schellenberg, D., C. Menendez, E. Kahigwa, J. Aponte, J. Vidal, M. Tanner, M. Mshinda, and P. Alonso.** 2001. Intermittent treatment for malaria and anaemia control at time of routine vaccinations in Tanzanian infants: a randomised, placebo-controlled trial. Lancet **357:**1471–1477.
29. **Schellenberg, D., C. Menendez, J. J. Aponte, E. Kahigwa, M. Tanner, H. Mshinda, and P. Alonso.** 2005. Intermittent preventive antimalarial treatment for Tanzanian infants: follow-up to age 2 years of a randomised, placebo-controlled trial. Lancet **365:**1481–1483.
30. **Shankar, A. H.** 2000. Nutritional modulation of malaria morbidity and mortality. J. Infect. Dis. **182**(Suppl. 1)**:**S37–S53.
31. **Stoltzfus, R.** 1997. Rethinking anaemia surveillance. Lancet **349:**1764–1766.
32. **Takyi, E. E.** 1999. Nutritional status and nutrient intake of preschool children in northern Ghana. East Afr. Med. J. **76:**510–515.
33. **Tréluyer, J. M., A. Roux, C. Mugnier, B. Flouvat, and B. Lagardere.** 1996. Metabolism of quinine in children with global malnutrition. Pediatr. Res. **40:**558–563.
34. **Uche-Nwachi, E. O.** 1998. Effect of intramuscular sulfadoxine-pyrimethamine on pregnant Wistar rats. Anat. Rec. **250:**426–429.
35. **Walker, O., A. H. Dawodu, L. A. Salako, G. Alvan, and A. O. Johnson.** 1987. Single dose disposition of chloroquine in kwashiorkor and normal children—

evidence for decreased absorption in kwashiorkor. Br. J. Clin. Pharmacol. **23:**467–472.
36. **Whitney, E., and S. N. Rolfes.** 1993. The water-soluble vitamins: B vitamins and vitamin C, p. 311–315. *In* E. Whitney and S. N. Rolfes (ed.), Understanding nutrition, 6th ed. West Publishing Company, St. Paul, MN.
37. **World Health Organization.** 2000. Severe falciparum malaria. Trans. R. Soc. Trop. Med. Hyg. **94**(Suppl. 1)**:**1–90.
38. **World Health Organization.** 2006. W. H. O. Child Growth Standards-Methods and Development. World Health Organization, Geneva, Switzerland.
39. **Wolday, D., T. Kibreab, D. Bukenya, and R. Hodes.** 1995. Sensitivity of *Plasmodium falciparum* in vivo to chloroquine and pyrimethamine-sulfadoxine in Rwandan patients in a refugee camp in Zaire. Trans. R. Soc. Trop. Med. Hyg. **89:**654–656.

Correspondence

Iron Deficiency and *Plasmodium falciparum* Infection During Pregnancy

To the Editor—Iron deficiency during pregnancy is particularly common in sub-Saharan Africa, where *Plasmodium falciparum* infection is another frequent cause of anemia. Because of the detrimental impact of anemia on the mother and the fetus, the World Health Organization universally recommends iron (and folate) supplementation, as well as malaria-prevention treatment, during pregnancy [1]. Iron supplementation has long been suspected to increase susceptibility to malaria [2], and a recent trial in malaria-holoendemic Zanzibar suggested that routine supplementation with iron and folate in children can result in an increased risk of severe illness and death [3]. A recent article by Kabyemela et al. [4] in the *Journal* raises additional concern. They report that, in Tanzanian pregnant women, iron deficiency was associated with an 80% reduction in the risk of placental malaria. This finding gives rise to the conclusion that iron supplementation for pregnant women in tropical regions may carry unrecognized risks and should be validated in large-scale clinical trials.

We reanalyzed data from a cross-sectional survey conducted in 1998 among 530 pregnant women in southern Ghana, an area of malaria holoendemicity [5]. Preceding the implementation of intermittent preventive treatment, almost 2 of 3 women attending antenatal care clinics in this area harbored *P. falciparum,* as evidenced by PCR analysis of peripheral blood samples, positive results of which almost invariably correspond to placental infection [6]. By use of the definition of iron deficiency (i.e., a ferritin level of <30 ng/mL, or <70 ng/mL if the C-reactive protein level was >8.2 ng/mL) and statistical analyses specified by Kabyemela et al. [4], we found that iron deficiency reduced the risk of *P. falciparum* infection in pregnant Ghanaian women by 33% (table 1). With regard to microscopically visible parasitemia only, the prevalence of infection among iron-deficient women was 21.5% (43 of 200), and the prevalence among women without iron deficiency was 38.8% (127 of 327) (odds ratio [OR], 0.43; 95% confidence interval [CI], 0.28–0.66; $P < .001$). Among iron-deficient women, geometric mean parasite densities tended to be lower (209 parasites/μL; 95% CI, 131–334) than those in women without iron deficiency (342 parasites/μL; 95% CI, 244–479; $P = .13$), and the proportion of women with submicroscopic infection (i.e., those in whom parasite levels are below the threshold of microscopic detection) was greater (70 [61.9%] of 113 vs. 93 [42.3%] of 220; $P < .001$). The reduction in the risk of PCR-confirmed *P. falciparum* infection was similar (even though, after stratification, it was not statistically significant) among primigravidae (OR, 0.46; 95% CI, 0.19–1.15) and secundigravidae (OR, 0.43; 95% CI, 0.18–1.06) but was only small among multigravidae (OR, 0.85; 95% CI, 0.51–1.40). This protective effect of iron deficiency was reversed in trimes-

Table 1. Logistic regression analysis of factors related to PCR detection of *Plasmodium falciparum* during pregnancy

Factor	No. of women	PCR positivity, % of women	OR (95% CI)	*P*	aOR (95% CI)	*P*
Gravidity						
1	130	73.1	1		. . .	
2	113	69.9	0.86 (0.47–1.55)	.59	0.91 (0.52–1.62)	.75
⩾3	287	56.1	0.47 (0.29–0.76)	.001	0.54 (0.34–0.86)	.009
Pyrimethamine in urine						
No	438	65.3	1		. . .	
Yes	92	53.3	0.61 (0.38–0.98)	.03	0.62 (0.39–0.99)	.04
Chloroquine in urine						
No	218	72.9	1		. . .	
Yes	312	56.4	0.48 (0.32–0.71)	.0001	0.53 (0.36–0.78)	.001
Iron deficiency[a]						
No	327	67.3	1		. . .	
Yes	200	56.5	0.63 (0.43–0.92)	.01	0.67 (0.46–0.98)	.04

NOTE. aOR, adjusted odds ratio; CI, confidence interval.

[a] Defined according to the criteria of Kabyemela et al. [4]. No data on ferritin levels were available for samples from 3 women.

ter 1 (OR, 2.70; 95% CI, 0.46–28.5; $n = 55$ women), pronounced in trimester 2 (OR, 0.56; 95% CI, 0.32–0.95), and also obvious in trimester 3 (OR, 0.65; 95% CI, 0.35–1.21). Moreover, iron deficiency conferred a similar degree of protection against *P. falciparum* infection among women with a normal hemoglobin type (OR, 0.62; 95% CI, 0.39–0.98) and those with the sickle cell trait (OR, 0.55; 95% CI, 0.18–1.64). Remarkably, this was not true for women with concurrent α-thalassemia (OR, 0.92; 95% CI, 0.46–1.82). In summary, we replicated and extended the observations of Kabyemela et al. [4] in an area of higher malaria endemicity (and lower iron deficiency prevalence) in West Africa.

However, a methodological problem remains. Serum ferritin level is considered the most useful laboratory measure of iron status, but a well-known limitation is its increase in patients with inflammation, including that due to malaria. The degree of elevation in the C-reactive protein level that invalidates use of ferritin level as the criterion of iron deficiency has not been accurately defined [7]. To address this problem, we used the following 3 definitions of iron deficiency in our initial report: a ferritin level of <12 ng/mL [8]; a ferritin level of <12 ng/mL, or ≤50 ng/mL if the C-reactive protein level was >6 ng/mL [9]; and a ferritin level of <30 ng/mL [10]. These definitions yielded iron deficiency prevalences of 5%, 18%, and 26%, respectively. For these classifications of iron status, concordance with the definition used by Kabyemela et al. [4] was 67%, 76%, and 88%, respectively. Repeated multivariate analysis with these initial categorizations of iron status revealed that the risk of *P. falciparum* infection was greatly reduced among women classified as iron deficient by the most robust definition (i.e., a ferritin level of <12 ng/mL; adjusted OR, 0.22; 95% CI, 0.09–0.54), among women with a ferritin concentration of <30 ng/mL (adjusted OR, 0.33; 95% CI, 0.22–0.50), but not among women with a ferritin level of <12 ng/mL, or ≤50 ng/mL if the C-reactive protein level was >6 ng/mL (adjusted OR, 1.01; 95% CI, 0.63–1.60) (table 1). We do not consider this latter finding as evidence of a spurious association. Rather, it underscores the difficulty in reliably defining iron deficiency in pregnant women exposed to *P. falciparum* and other infectious diseases, which may eventually mask actual associations. Kabyemela et al. [4] call for clinical trials to guide the use of iron supplementation in pregnant women living in malaria-endemic regions. Our data support this and, additionally, highlight the need for evaluating diagnostic means of determining iron deficiency in this risk group.

Acknowledgments

We thank the participating women and the midwives and administrative staff at Agogo Hospital.

Ina Danquah,[1] George Bedu-Addo,[2] and Frank P. Mockenhaupt[1]

[1]Institute of Topical Medicine and International Health, Charité–University Medicine Berlin, Berlin, Germany; and [2]Department of Medicine, School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

References

1. WHO/UNICEF/UNU. Iron deficiency anaemia: assessment, prevention and control: a guide for programme managers. Document WHO/NHD/01.3. Geneva, Switzerland: WHO, **2001**.
2. Oppenheimer SJ. Iron and its relation to immunity and infectious disease. J Nutr **2001**; 131(2S-2):616S-633S.
3. Sazawal S, Black RE, Ramsan M, et al. Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomised, placebo-controlled trial. Lancet **2006**; 367:133–43.
4. Kabyemela ER, Fried M, Kurtis JD, Mutabingwa TK, Duffy PE. Decreased susceptibility to *Plasmodium falciparum* infection in pregnant women with iron deficiency. J Infect Dis **2008**; 198:163–6.
5. Mockenhaupt FP, Rong B, Günther M, et al. Anaemia in pregnant Ghanaian women: importance of malaria, iron deficiency, and haemoglobinopathies. Trans R Soc Trop Med Hyg **2000**; 94:477–83.
6. Mockenhaupt FP, Ulmen U, von Gaertner C, Bedu-Addo G, Bienzle U. Diagnosis of placental malaria. J Clin Microbiol **2002**; 40: 306–8.
7. Cook JD. Diagnosis and management of iron-deficiency anaemia. Best Pract Res Clin Haematol **2005**; 18:319–32.
8. Cook JD, Skikne BS. Iron deficiency: definition and diagnosis. J Intern Med **1989**; 226: 349–55.
9. Kuvibidila S, Yu LC, Ode DL, Warrier RP, Mbele V. Assessment of iron status of Zairean women of childbearing age by serum transferrin receptor. Am J Clin Nutr **1994**; 60:603–9.
10. van den Broek NR, Letsky EA, White SA, Shenkin A. Iron status in pregnant women: which measurements are valid? Br J Haematol **1998**; 103:817–24.

Potential conflicts of interest: none reported.

Financial support: Charité (grants 99-640 and 99-641); Sonnenfeld Foundation, Berlin (grant to I.D.).

Reprints or correspondence: Ina Danquah, Institute of Tropical Medicine and International Health, Charité–University Medicine Berlin, Spandauer Damm 130, 14050 Berlin, Germany (ina.danquah@charite.de).

The Journal of Infectious Diseases 2008; 198:1573–4

0022-1899/2008/19810-0022$15.00
DOI: 10.1086/592449

SELBSTÄNDIGKEITSERKLÄRUNG

„Ich, Ina Danquah, erkläre, dass ich die vorgelegte Dissertation mit dem Thema: „Die Rolle des Ernährungszustandes bei Patienten mit Diabetes mellitus Typ 2 und Malaria in sub-Sahara Afrika“ selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt, ohne die (unzulässige) Hilfe Dritter verfasst und auch in Teilen keine Kopien anderer Arbeiten dargestellt habe.“

Datum Unterschrift

DANKSAGUNG

Für die erfolgreiche Erstellung dieser Arbeit bedanke ich mich herzlich bei einer Vielzahl von Menschen in meinem Umfeld.

Dazu gehört an erster Stelle mein Doktorvater Prof. Dr. med. Frank P. Mockenhaupt (Institut für Tropenmedizin und Internationale Gesundheit, Charité). Für das mir entgegengebrachte Vertrauen, die Geduld bei der wissenschaftlichen Anleitung und die hilfreiche Kritik an meiner Arbeit bin ich ihm sehr verbunden. Weiterhin möchte ich der Leitung des Instituts für Tropenmedizin und Internationale Gesundheit, Prof. Dr. med. Ulrich Bienzle und Prof. Dr. med. Gundel Harms, für die Vergabe meines Promotionsthemas und ihre konstante Unterstützung bei der wissenschaftlichen Arbeit danken.

Besonders möchte ich die verlässliche und freundliche Kooperation mit Dr. George Bedu-Addo (Komfo Anokye Teaching Hospital, Kumasi, Ghana) und mit dem Team der *Kumasi Diabetes und Hypertonie Studie* würdigen. Ebenso wichtig waren die kompetenten Beratungsgespräche zu statistischen Herausfordeurngen mit Dr. rer. nat. Ekkehart Dietz (Institut für Biometrie und klinische Epidemiologie, Charité).

Hervorzuheben ist auch die Unterstützung durch ein 2-jähriges Doktoranden-stipendium der Sonnenfeld-Stiftung, Berlin.

Ich danke auch meinen Freunden Sandra Brunsbach, Ines Middelbeck, Christiane Ehrig, Anne-Kathrin Illner, Marcus Lukas und Ralph Kattenbach, die mich durch die Höhen und Tiefen dieser Arbeit begleitet haben.

Nicht zuletzt haben meine Eltern, Antje und Peter Schöttle, durch ihre mentale Unterstützung und Hilfe bei der Kinderbetreuung maßgeblich zum Erfolg dieser Arbeit beigetragen. Vor allem möchte ich meinen Kindern Jacob und Simon danken, die mir stets die nötige Kraft und Motivation für das Gelingen der Promotion gegeben haben.

Berlin, September 2010

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.